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Journal of DENTAL RESEARCH

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This *Journal* is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The *Journal* publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this *Journal*.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

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Editorial Views and News

In the last week of this year, Section Nd of the American Association for the Advancement of Science has two interesting symposia organized in New York City. This meeting, as in the past, is co-sponsored by the International Association for Dental Research. Nd Section (Dentistry) Vice-President and Chairman is Joseph L. T. Appleton; Reidar F. Sognnaes is Secretary, and Earl O. Butcher is Program Chairman of this meeting. (In a historical sense it is of interest to note that the first meeting of the Nd group, then only a subsection, was held January 4, 1936. However, the American College of Dentists had affiliated with the A.A.A.S., holding joint symposia with other sections since December 29, 1932.)

December 30, 1960.—Symposium on "Fundamentals of Keratinization," Section Nd (Dentistry) sponsored by American College of Dentists, American Dental Association, and International Association for Dental Research, North American Division.

9:00 A.M.: EARL O. BUTCHER, *Moderator.*

"Mechanism of Keratinization," A. Gedeon Matoltsy, Research Professor, Department of Dermatology, University of Miami.

"The Histochemical Distribution of Sulfhydryls and Disulfides in Vertebrate Keratins," R. J. Barrnett, Associate Professor of Anatomy, Yale University, School of Medicine, and Reidar F. Sognnaes, Dean, School of Dentistry, University of California, Los Angeles.

"Keratinization of Whole Skin and Isolated Epidermis in Vitro," George Szabo, Associate in Anatomy, Harvard Medical School.

"Keratinization as Seen with the Electron Microscope," J. A. Rhodin, Professor of Anatomy, and E. J. Reith, Assistant Professor of Anatomy, New York University, School of Medicine.

"The Effects of Vitamin A on Keratinizing Epithelia," Howard A. Bern and Donald J. Lawrence, Department of Zoölogy and its Cancer Research Genetics Laboratory, University of California, Berkeley.

"Effects of Vitamin A on Keratinization in the 'A'-Deficient Rat," J. P. Parnell and B. Sherman, Department of Anatomy, State University of New York.

2:00 P.M.: REIDAR F. SOGNNAES, *Moderator.*

"The Effect of Environment on the Physical Characteristics of the Cornified Epithelium," Irvin H. Blank, Associate in Dermatology, Harvard Medical School.

"Keratinization of the Oral Mucosa," Julia Meyer, Associate Professor, Division of Oral Pathology, College of Dentistry, University of Illinois.

"Keratinization in Dental Cysts," Jens J. Pindborg, Professor of Oral Pathology, The Royal Dental College, Copenhagen, Denmark.

"The Extracellular Position of Enamel," M. L. Watson, Associate Professor of Pathology and Radiation Biology, University of Rochester.

"The Chemistry of the Protein Matrix of Enamel," K. A. Piez, National Institutes of Health, Bethesda, Maryland.

December 29, 1960.—Symposium on "Career Opportunities in Medicine and Dentistry."

9:00 A.M.: "Introductory Remarks," Charles V. Reichart, O.P., Ph.D., Presiding Head, Biology Department, Providence College A.E.D. National Treasurer.

9:10 A.M. "Physician Needs for the Future," William H. Steward, M.D., Chief, Division of Public Health, Education, and Welfare.

9:40 A.M.: "Dentist Needs for the Future," Shailer Peterson, Ph.D., Secretary, Council on Dental Education, American Dental Association.

10:10 A.M.: Panel Discussion—"Opportunities in Dentistry." RAYMOND J. NAGLE, D.D.S., *Moderator,* Dean, New York University College of Dentistry. Joseph A. Cuttita, D.D.S., Chairman, Admissions Committee, School of Dental and Oral Surgery, Columbia University; Merritte M. Maxwell, D.D.S., Dean, Seton Hall College of Medicine and Dentistry; W. A. Wilson, D.D.S. Dean, School of Dentistry, Fairleigh Dickinson University; J. Howard Oaks, D.D.S., Assistant in Charge of Student Affairs, Harvard School of Dental Medicine; Lester W. Burket, D.D.S., Dean, the Thomas W. Evans Museum and Dental Institute School of Dentistry, University of Pennsylvania; Louis Herman, D.D.S., Chairman, Admissions Committee, Temple University School of Dentistry.

11:00 A.M.: Panel Discussion "Opportunities in Medicine." JAMES O. PINKSTON, M.D., *Moderator,* Associate Dean, Downstate Medical Center, State University of New York. George Perera, M.D., Assistant Dean, College of Physicians and Surgeons, Columbia University; Lawrence W. Hanlon, M.D., Associate Dean, Cornell University Medical College; Stuart Sevenson, M.D., Chairman, Department of Pediatrics, Seton Hall College of Medicine and Dentistry; James C. Niederman, M.D., Assistant Professor of Epidemiology and Preventive Medicine; Member, Pre-Advisory Committee of Yale College; Sol J. Farber, M.D., Assistant to the Dean, Associate Professor of Medicine, New York University College of Medicine.

12:15 P.M.: Luncheon. Address by I. S. Ravdin, M.D., Vice-President for Medical Affairs. Afternoon conferences with admissions officials. Visit to medical and dental schools.

—F. J. O.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

THIRTY-NINTH ANNUAL MEETING, MARCH 23, 24, 25, AND 26, 1961
HOTEL STATLER, BOSTON, MASSACHUSETTS

Persons interested in dental research and related fields are invited to attend. A modest registration fee will be charged to help defray costs of the meeting. Programs and a limited number of copies of the Preprinted Abstracts will be available at the meeting for *non-member* registrants. Members are asked to bring with them the programs and Preprinted Abstracts which will be mailed to them before the meeting.

First International Conference on Oral Biology

Abstracts of Papers Presented

NEW YORK CITY, SEPTEMBER 7, 8, 9, 1959

Compiled by NED B. WILLIAMS and HAMILTON B. G. ROBINSON

The first International Conference on Oral Biology was sponsored by the International Association for Dental Research and the American Dental Association with the aid of a grant of $30,000 from the Colgate-Palmolive Company. The planning was placed in the hands of a committee of Drs. Ned B. Williams, Chairman, Basil G. Bibby, D. Walter Cohen, Josse E. de Wever, James A. English, Eli D. Goldsmith, John W. Hein, Seymour J. Kreshover, and Hamilton B. G. Robinson.

The conference was held in New York City on September 7–9, 1959, immediately preceding the centennial meeting of the American Dental Association. The program consisted of six sessions, two each day, and a dinner session. There were 55 speakers and discussers, including 8 from overseas, 49 invited representatives from the dental schools in North America, and 201 guests from 15 different countries.

At the dinner session, which was attended by 118 individuals, Dr. Carl Sebelius presented a beautifully illustrated lecture on his recent experiences around the world as the World Health Organization's dental officer. Dr. Hamilton B. G. Robinson, president of the International Association for Dental Research, introduced the international guests, including representatives of the Fédération Dentaire Internationale, and expressed appreciation to the sponsor, the Colgate-Palmolive Company.

I. ENAMEL. SEPTEMBER 7, 1959. MORNING

1. CHEMISTRY OF THE ENAMEL SURFACE.—*Finn Brudevold, Forsyth Dental Dispensary, Boston, Massachusetts.* While there is little difference in the concentration of calcium and phosphate in surface and subsurface enamel, marked differences occur with regard to certain other enamel constituents. F, Zn, Pb, and to a lesser extent Fe, Ag, Mn, Si, and Sn are present in greater concentrations in the outer than in the inner portions of the enamel. Carbonate, Na, and Mg follow an opposite distribution pattern and increase in concentrations from the surface inward. Some elements, including Cu and Sr, occur in similar concentrations in surface and subsurface enamel. Analysis of organic constituents, i.e., nitrogen, citrate, and lactate, show greater concentrations in surface enamel than in adjacent underlying enamel. There seems to be less water in surface than in subsurface enamel. The composition of surface enamel varies greatly in teeth from different geographical regions. The concentration of F in outer enamel increases almost linearly with increase in F in the drinking water. The increase in F of surface enamel with age is more marked at low than at high concentrations of F in the drinking water. In addition to Ca and P, the constituents of surface enamel occurring in greatest molar concentrations include carbonate, Na, F, Mg, and Zn. Citrate, Al, Sr, Pb, Mn, Cu, Si, Ag, Fe, and Sn are present only in concentrations less than 10 micromoles per gram and are therefore less likely to affect its physical properties.

2. CHEMICAL ACTION OF EXTERNAL FLUIDS ON ENAMEL.—*S. Yngve Ericsson, Dental School, Stockholm, Sweden.* External fluids, by definition, comprise saliva, ingested fluids, and regurgitated gastric contents. The permeability of the enamel to water and water-soluble substances seems to be confined to structures of comparatively low min-

eral content. The dentino-enamel junction forms a partial barrier to this penetration. Of primary importance is the solubility of the enamel substances, especially that of the calcium phosphate. Several efforts have been made to obtain a mathematical expression of the solubility of hydroxyapatite. However, solubility products for hydroxyapatite or tracalcium phosphate given by the physical chemists are not directly applicable to the saliva. At physiological pH values the solubility of calcium phosphate in saliva is much higher than in CO_2-free salt solutions of comparable ionic strength. There is considerable evidence that this difference depends to a large extent on the CO_2 content of the saliva. Freshly secreted mixed saliva is apparently supersaturated with calcium phosphate. Some calcium may, however, be bound by the mucoid, making the supersaturation more or less fictitious. Splitting of the mucoid may liberate calcium and increase the tendency to precipitation of calcium phosphate on the tooth surfaces. Calcium phosphate contained in foods or added to foods may similarly play a protective role for the enamel surface. The calcium carbonate of the enamel is more readily dissolved in weak acids than the phosphate, and the carbonate content of the enamel surface therefore decreases with age. The possible role of chelating substances may be exemplified by citrate. There is no measurable effect on the calcium phosphate solubility from the citrate concentrations remaining in the saliva 2 minutes after the ingestion of citrate-rich fruits or beverages. The erosive effect of citrate should thus be largely limited to the times of ingestion. Speculations on the role of weaker chelating substances should be restricted as long as no quantitative data or calculations exist. Fluoride is most important among the ions exerting a solubility-decreasing effect on the enamel surface. An increased resistance of enamel to the action of weak acids is produced by fluoride supplied with water, probably also by the low concentration of fluoride in the continuous flow of saliva. Little is known of the role in saliva and the effect on the enamel of other ions which decrease the solubility of enamel salts, such as lead, tin, ferrous iron, oxalate, phytate, and others. The protective action of the salivary mucoid and of the salivary buffer capacity was pointed out.

3. Certain Aspects of the Submicroscopic Structure of Enamel.—*Otto R. Trautz, New York University, New York.* The X-ray diffraction patterns of dental enamel identify its only crystalline component as apatite. They show the orientation of the crystallites in the enamel rods, and from the intensity profiles of the diffraction lines the dimensions of the crystallites are determined. A cut across one enamel rod may cut across five to ten thousand crystallites. The electron microscope, on the other hand, shows the dimensions of the particles, which may contain several or many crystallites cemented together without coherence of their lattices. Impurities (e.g., $CO_3^=$, $HPO_4^=$) reduce the thickness of the crystallites, which are elongated parallel to their *c*-axes, and distort the lattice planes. Increasing amounts of these impurities will increase the distortion until an amorphous precipitate results, evidenced by a diffuse diffraction band. This we found in various tissues as well as in vitro experiments. The phosphate likewise disturbs the crystallization of the carbonate. From solutions containing appreciable amounts of phosphate at a neutral pH we have never obtained crystallized calcium carbonate, and we do not expect to find it in tissues together with apatite. However, the presence of a more or less amorphous material together with apatite is possible. The impure apatite is more reactive than the well-crystallized pure apatite. NaF (2 per cent) solution attacks the enamel and forms CaF_2. The effect of NaF solution on various crystallized calcium salts (calcite, monetite, brushite, gypsum, OH apatites, F apatites, francolite, bone, dentin, enamel) was studied, identifying the solid reaction products by X-ray diffraction. From the results of such experiments we conclude that topical application of NaF, first of all, transforms the accessible acid-soluble carbonate and secondary phosphate into the acid-insoluble CaF_2. Second, on the accessible surfaces of the OH apatite crystallites OH^- may be exchanged for F^-, which, however, does not diffuse into the crystal. Only by solution and new crystallization can F get into the crystals. The enamel is fortified by fluoride against demineraliza-

tion particularly along the same diffusion paths as those used by the demineralizing agents.

4. EXPERIMENTAL MODIFICATIONS OF THE ENAMEL SURFACE.—*Hans R. Muhlemann, University of Zurich, Switzerland.* The in vitro attack of several acid buffers and of Na_2-EDTA on intact enamel has been investigated simultaneously in replicates and in ground sections. Surface etching and white-spot formation were compared with the amount of phosphorus released during enamel demineralization. The etching pattern by acid buffers can be distinguished from the EDTA attack. Agitated aqueous pH 4 buffers produced after 30 minutes definite breaks in the surface continuity: Addition of sucrose to the buffers inhibited the enamel solution rate by 50 per cent but did not alter the pattern of microscopic etching of the enamel surface. The addition of 1–5 per cent gelatin to the demineralizing solution, the pH being adjusted to 3.85, inhibited the removal of phosphorus also by approximately 50 per cent. However, instead of a surface etching, the formation of subsurface demineralization—white-spot formation—occurred.

II. BIOCHEMICAL AND NUTRITIONAL FACTORS IN DENTAL CARIES
SEPTEMBER 7, 1959. AFTERNOON

5. NUTRITIONAL FACTORS IN DENTAL CARIES.—*H. D. Cremer, Giessen University, Giessen, Germany. Nutritional factors,* characterized by the systemic effect of absorbed food upon the developing tooth, *and dietary factors,* which include the environmental effects of foodstuffs, nutrients, and dietary habits, should be differentiated. The report on nutritional factors has to be divided into three parts: (1) To what extent is it possible to compare caries in animals with caries in human beings and to draw conclusions from results given by animal experiments on human caries? A short description of the new microscopic method for caries diagnosis after short-term experiments was given. (2) What kind of tests have we for deciding whether any nutritional or dietary influence is working. If nutritional factors are to influence subsequent caries, it is likely that they will do so during the formative period of the teeth. There are different possibilities to test such changes of the teeth: (*a*) changes in the morphological structure, (*b*) changes in the chemical structure, (*c*) changes in metabolic processes in the tooth tissue, and (*d*) factors to be tested which are working before eruption of the teeth into the oral cavity. (3) Which components of the diet, which nutrients, which factors in our nutrition are supposed to be working as nutritional factors in dental caries?

The following nutritional factors have been mentioned: (*a*) vitamins (vitamin D and other vitamins); (*b*) minerals (ash of natural diets, fluorine, and other trace elements); (*c*) certain foodstuffs and food constituents (sugar; potatoes, white bread, and oats; yeast and cocoa beans; processed foodstuffs; cooking of the diet). The report presents only one part of the correlations between caries, food, and nutrition.

6. DIETARY FACTORS IN DENTAL CARIES.—*O. Backer-Dirks, Laboratory of Hygiene, Utrecht, Holland.* The dietary influence factors are mainly twofold: (1) posteruptive influences on the tooth ("posteruptive maturation") and (2) effect of diet on the tooth environment. The first influence has received more and more attention during the last 10 years. In the period just after eruption, ion exchanges are at a high level equal to that before eruption. Crystallites increase in size, etc. Animal experiments have proved definitely the importance of this period. The dietary factors promoting a good "maturation" seem to be the same as for a caries-inhibiting environment. The influence of the war diet in Europe seems—at least partly—the result of beneficial conditions during posteruptive maturation. Fluoride also plays an important part in this period. It has been proved that bacteria and carbohydrate retention in the mouth are necessary for caries initiation. The bacterial flora for each site in the mouth is partly determined by factors we cannot influence (saliva, tooth and tooth position, mucous membrane, etc.) and partly by the kind and amount of substrate introduced into the

plaque by the diet. The effect of a newly introduced substrate will depend partly on the balance between acidogenic, acid-consuming, and proteolytic bacteria and partly on the effective plaque thickness, buffer capacity, and flow rate of saliva, etc. For each surface these conditions will be different.

The time during which fermentable sugar is present in the plaque seems to be of paramount importance in the caries initiation. The conditions governing this time are (1) the individual's mouth physiology (cleansing function, saliva properties, bacterial flora, etc.); (2) the physical state of the diet; (3) the physical state of the carbohydrates in the diet (e.g., tough or hard, solid, in solution, etc.); (4) the chemical form of the carbohydrate (e.g., mono-, di-, or polysaccharoids, fermentability, etc.); and (5) the frequency of eating. Animal experiments and observations in human beings (experiments and dietary surveys) have proved the importance of each of these factors. Proteins, fat, and other dietary factors need more attention. Animal experiments point these out as protective foods. The period just after eruption is of great importance for the future of the tooth. There is reason to believe that caries-conducive factors are especially dangerous in this period. The most important factor in caries initiation is the time during which fermentable sugars are present in the plaque. Eating between meals —especially sweets in all forms—increases this time materially. Detergent food will decrease this time. The only known element which has posteruptively a definite beneficial effect is fluorine.

7. PHOSPHORUS AND DENTAL CARIES.—*Robert S. Harris, Massachusetts Institute of Technology, Cambridge, Massachusetts.* Almost without exception, investigators have noted decreases in the incidence of severity of experimental caries when phosphorus was added to caries-producing diets. The data from these studies vary quantitatively primarily because of differences in experimental conditions. However, it does appear that phosphates, which are more soluble in water, may be more effective as cariostatic agents and that metaphosphoric acid is the most effective of all. It is not yet clear whether this effect is local, systemic, or both. It is difficult to believe that there is no local action on the surface of the tooth. It is quite possible that a significant cariostatic effect cannot be correlated with changes in the structure and composition of tooth enamel, because the effect is due to surface changes of the enamel which cannot be detected by the usual techniques of analysis. A delicate separation of the surface layers from the disproportionately large mass of subsurface enamel may be necessary to establish this point. Studies on tube feeding and on the blood indicate possible systemic effects. Thus it is probable that the effect is both local and systemic, but we must wait for convincing proof of this. It does appear that the level of phosphorus (and calcium) in the diet has a critical effect on the development of experimental caries. The Ca:P ratio does not seem to be critical unless the level of Ca or of P in the diet is limiting, just as in the development of experimental rickets in rats.

8. RELATION OF TRACE ELEMENTS TO DENTAL CARIES.—*Walter C. Hess, Georgetown University, Washington, D.C.* Analyses have revealed the presence of many metals in trace amounts in bones and teeth. Suggestions have been made repeatedly that trace metals may play an important part in the calcification of hard tissues. The evidence to support these suggestions is meager and difficult to interpret. Such metals as magnesium, manganese, and molybdenum, that are required for certain enzyme activities, are probably necessary for calcification. Other metals, such as vanadium, copper, selenium, and beryllium, while toxic at certain levels, have not been shown to have beneficial physiologic effects on calcification. Studies employing the ash obtained from whole diets have been equivocal in their results.

9. QUESTIONS RAISED BY THE INFECTIOUS AND TRANSMISSIBLE NATURE OF EXPERIMENTAL CARIES.—*Paul H. Keyes, National Institute of Dental Research, Bethesda, Maryland.* Under proper experimental conditions, one can show that dental caries is an

infectious and transmissible disease in both hamsters and rats. However, to demonstrate this, three sets of conditions must be present simultaneously: (1) the "cariogenic" flora must be acquired by animals not originally possessing it; (2) the teeth must be in a susceptible state, e.g., at a suboptimal stage of maturation; (3) the diet must have the specific chemical and physical properties necessary to induce activity. In all probability, under general laboratory conditions, young animals acquire most of their alimentary microbiota from the feces of the mother animal. If the female carries organisms with a "cariogenic" potential, these will also be transmitted. It is also possible to acquire the flora from other animals or by modes of cross-infection; however, it should be emphasized that present findings suggest that micro-organisms with a cariogenic potential for one strain and species may not be equally pathogenic for another. For example, it has not been possible to induce caries in "non-infected" hamsters by contaminating them with rats; nor has it been possible to increase caries activity in black rats, which have a low "susceptibility," by caging them with highly active Osborne-Mendel animals. In addition to these findings, it has been shown in hamsters that the dietary history of the mother and her own caries experience may be independent of the susceptibility to caries of her offspring. A "susceptible" (infected) mother will not develop caries unless maintained on a cariogenic diet; but if she harbors a cariogenic flora, her offspring will be caries-active if a cariogenic diet is provided. Conversely, a female not harboring a cariogenic flora will not develop caries even when maintained on a cariogenic diet, and her offspring will remain caries-free unless deliberately infected by exposure to caries-active animals or by the administration of feces from caries-active animals. Infected hamsters can be made "caries-resistant" by a brief exposure to antibiotics, such as penicillin or erythromycin, which eliminate the cariogenic microflora, and the offspring of females treated in this way remain caries-free unless they are reinfected. All these findings direct attention to the alimentary canal microbiota and the anal-oral cycle of laboratory animals. How does this microbiota differ between animals? What are the various experimental conditions which affect it? How do dietary alterations increase or decrease the level of various types of micro-organisms present, including those associated with caries? How do constitutional factors influence the diet-flora interrelationship? Are there antagonistic and synergistic microbical relationships involved? How can the "cariogenic" flora be equalized for certain types of critical experiments? These and many other questions need to be answered to place experimental caries research on a sound scientific basis.

III. ORAL SOFT TISSUES. SEPTEMBER 8, 1959. MORNING

10. BIOCHEMISTRY OF INFLAMMATION.—*Bernard K. Forscher, University of Kansas City, Kansas City, Missouri.* An experimental method for the chemical study of acute inflamamtion has been presented and some of the chemical changes which characterize the inflammatory response described. Of major interest at this time are the effects of systemic conditions on the chemistry of the local inflammatory response. It was shown that both diabetes and increased age in the experimental animals markedly affected the curves for organic acid–soluble phosphate and for DNA. The significance of the glycogen curves as markers for leukocyte influx was discussed. It appears that, while both alloxan diabetes and increasing age have a deleterious effect on the acute inflammatory response, the mechanisms of these two effects differ when considered from a chemical point of view. Some consideration was given to the problem of biochemical mediators of the inflammatory response. Several of the current theories in this field were reviewed, and it appears that there are insufficient data at the present time to select conclusively a definite explanation from among the various possibilities.

11. THE NATURE AND MECHANISM OF TISSUE DESTRUCTION.—*Stig. D. Schultz-Haudt, Norwegian State Dental School, Oslo, Norway.* A series of collagen determinations on healthy and chronically inflamed human gingivae have been analyzed. According to these measurements, there is no dramatic breakdown of collagen in chronic in-

flammation. The apparent breakdown, as interpreted from histologic sections, seems to be due to a disorganization of the fibers and a failure of precipitating the fibers from the precursors. The available evidence indicates that these alterations are secondary to changes in the ground-substance polysaccharides. These changes, in turn, may involve the state of aggregation of the polysaccharide-protein complexes (depolymerization), as well as quantitative differences (as compared with the normal situation) in the synthesis by the cells of hyaluronic acid and chondroitin sulfuric acids. Changes of the kind, which will be reflected in the status of the collagenous fibers, may be due to bacterial activity on the gingiva or in the gingival crevice.

12. The Meaning of Some Oral Symptoms: Oral Tissues as Indicators of Cell Oxidations.—*Philip Person, Veterans Administration Hospital, Brooklyn, New York.* Cytochemical and biochemical experiments from the speaker's laboratory were reported, dealing with differences in the aerobic oxidative behavior of tongue, salivary glands, and gingiva. In the first group of experiments, the synthesis of indophenol blue (Nadi reaction) by fresh-frozen slices of tongue, salivary glands, and gingiva was described. The outstanding feature of these studies was the demonstration that salivary gland ducts and tubules possess a greater oxidative capacity than do the acini in all the major salivary glands in the rat. The reproducible variations in oxidative capacity of different cell areas of human gingival tissue were also described. In these latter experiments the most interesting finding was the existence of an oxidation potential in keratin. This is the first time that such an oxidative capacity has been demonstrated in what has hitherto been considered an inert protective protein structure. The possible significance of this finding to disulfide bond formation during keratogenesis was briefly discussed. Biochemical determinations of human gingival oxygen consumption were performed in conjunction with the above cytochemical studies. The cytochrome oxidase activity in most runs was between 17 and 25 (Q_{O2}/mg N). On a dry-weight basis these values would be between 3 and 4, confirming the findings of earlier workers to the effect that oxygen consumption by gingiva is very low compared with other tissues. In this, gingiva behaves like cartilage, skin, lens, and cornea. Therefore, a search was made for possible causes of the low oxygen utilization by the above tissues, using cartilage as a beginning. First, it was shown that cartilage homogenates added to active cytochrome oxidase preparations inhibited oxygen utilization. Next, isolated components of cartilage were examined. Collagen did not possess such inhibitory properties. Anionic mucopolysaccharides, however, did possess inhibitory action when added to either fresh or partially purified heart-muscle oxidase preparations. Chondroitin sulfate, heparin, and other similar materials exhibit this property. For the gingiva we have found in some, but not all, experiments that homogenates of gingiva also may inhibit oxygen utilization of active heart-muscle preparations. The above experiments and others have led the author provisionally to explain the low oxygen utilizations by gingiva, cartilage, etc., as being due in part to their high content of anionic macromolecular mucopolysaccharides. The view is expressed that substances such as chondroitin sulfate and other compounds of the class of polyanionic macromolecules, in addition to serving as structural components of tissues, may also exert very significant metabolic regulatory functions. The author's own experiments and those in other laboratories, in which enzymes such as ribonuclease have been shown to be inhibited by such compounds, are marshaled in support of the above view.

13. Epithelial Reactions in Periodontal Disease.—*B. Cohen, Royal College of Surgeons, London, England.* Discussion was based largely on histologic findings from experiments instituted in an attempt to identify the initial lesion in periodontal disease. As a result of this investigation, the concept was put forward that periodontal disease commences with a failure of the epithelial integument to protect the underlying tissues of the periodontium. The commonest site of epithelial failure is the reduced enamel epithelium rather than the stratified squamous epithelium of the oral mucosa.

During the course of investigating the distribution of enamel epithelium, periodontally it was found that the interdental gingival septum between recently erupted teeth which are in contact with one another is covered not by oral epithelium but entirely by enamel epithelium. This enamel epithelial covering is derived from the juxtaposition back to back of the enamel epithelia covering the interproximal surfaces of the contiguous teeth. Furthermore, the outline of the interdental gingival septum does not follow the outline of the cemento-enamel junction, as had previously been believed; instead, it constitutes a depression between two peaks, the peaks being the stratified squamous epithelium forming the papillae which occupy the buccolabial and linguopalatal embrasures. Viewed in buccolingual section, the septum therefore has the outline, not of a papilla as usually described, but of a col ("col" being the expression used in mountaineering for a ridge-shaped depression lying between two peaks). The reason for this configuration is explicable by a study of epithelial structure during the course of eruption of teeth in contact with one another. The interdental septum constitutes an area of maximum vulnerability in the period immediately after tooth eruption. If and when oral epithelium grows through beneath the contact point from papilla to papilla, this area becomes normally protected. Until this happens, however, or if, as in certain circumstances, it is prevented from happening, the initiation of periodontal disease is likely. The individual properties of oral and enamel epithelia, which led to the belief that the latter is more vulnerable, and the reasons for the belief that damage on the buccal or lingual is less serious than that sustained interdentally were discussed. It was pointed out that the clinical application of this work is that incipient periodontal disease can be recognized and can be prevented in the period immediately following tooth eruption.

IV. ORAL SOFT TISSUES. SEPTEMBER 8, 1959. AFTERNOON

14. THE ROLE OF ASCORBIC ACID IN PERIODONTAL TISSUE.—*Jens Waerhaug, Norwegian State Dental School, Oslo, Norway.* The examination of four monkeys living on vitamin C–deficient diet gave the following results: Obvious clinical signs of scurvy appeared after approximately 90 days. After 120 days, increased mobility of the teeth was observed. Concomitant with the loosening of the teeth, the gingivae became flabby and bleeding. In the histologic sections from one animal which had lived on the vitamin C–deficient diet for 275 days, the following observations were made: The periodontal membrane was widened, sometimes extremely so. The alevolar crest was usually of normal height. The collagenous fibers of the periodontal membrane were nearly completely broken down except for scattered areas. It was a constant finding that some Sharpeys fibers were found to be attached to the cementum, whereas no such fibers were found on the bone side. It was also found that the principal fibers of the periodontal membrane just below the epithelial cuff were unusually well maintained, even in cases where most of the gingival fibers were lacking. This is probably the reason why downgrowth of epithelium occurred only in exceptional cases. The increased mobility apparently did not lead to pocket deepening, since the periodontal fibers just below the epithelial cuff prevented downgrowth of this. The clinical appearance of the periodontal structures in severe scurvy resembles in many respects that of ordinary periodontal disease in man, being characterized by increased mobility and bleeding gums. However, the histologic picture is different. In severe scurvy the damage of the periodontal membrane is generalized, whereas in periodontitis it is localized to the area below the deepened pocket. Increased mobility in scurvy is facilitated by the generalized destruction of the collagen of the periodontal membrane and the resorption of the inside of the alveolar wall, whereas in periodontitis it is facilitated by the pocket deepening and localized destruction of the periodontal membrane.

15. RECURRENT MIKULICZ'S APHTHAE TREATED WITH TOPICAL HYDROCORTISONE HEMISUCCINATE SODIUM.—*B. E. D. Cooke, Department of Dental Medicine, Guy's Hospital, London.* A recent favorable report of the use of topical hydrocortisone

hemisuccinate in recurrent aphthae was thought to be encouraging enough to merit a controlled trial of this form of treatment. A double-blind controlled clinical trial was undertaken to assess the relative value of tablets containing 2.5 mg. of hydrocortisone hemiscuccinate with a lactose base and an inert preparation of the lactose base alone to patients suffering from these ulcers. Patients were given four tablets a day to take when the ulcers were present and two tablets a day when they were not and were asked to allow the tablets to dissolve in the region of the ulceration. This meant that most patients were, in fact, taking four tablets a day, apart from four patients who had complete remissions and who then took only two a day. The trial involved 17 patients (8 male, 9 female), all with a long history of the same type of severe recurrent oral ulceration, often known as "Mikulicz's aphthae." At the start, every patient kept the record for at least a month and then was allotted tablet A or B from a list of random numbers. During the trial it was not known either to patients or to the investigator which of the two tablets contained the hydrocortisone hemisuccinate. Two figures lent themselves to the most accurate measurement, namely, the number of new ulcers and the number of ulcer-days in a given period. By "ulcer-days" is meant the product of the number of ulcers and their duration in days, e.g., two ulcers occurring at the same time and each lasting a week are recorded as 14 ulcer-days. This unit gives a measure of the amount of distress experienced by the patient. There is a much better clinical response when the patients were being treated with the drug, as compared with the inert tablet, with the difference being greater when the placebo was taken first. When considering all the patients together, results shown to be statistically significant at the 5 per cent level reveal that, compared with the placebo, there is a 50 per cent reduction by the drug of ulcer-days and of new ulcers per patient per 8-week period. That psychological factors play an important part is suggested not only by the difference between the effect when the active preparation preceded the placebo and vice-versa but also by the varying response between patients. Since this therapy in no way cures or completely prevents these troublesome ulcers, four tablets a day for 8 weeks would appear to be enough to help a patient during a particularly severe bout of ulceration. The resultant relief from pain may induce such a feeling of well-being that the ulceration may remain at a less severe level for a month or two, after which another period of therapy could be tried if desired.

16. The Effects of Protein Deprivation on Periodontal Tissues.—*Max Shapiro, Beverly Hills, California.* The study was designed to test the effects of a diet containing 10 per cent protein, compared with a diet of normal protein requirements (25 per cent). In addition, a study was made on the effects of gamma radiation in these normal and protein-deficient animals. The increment in body weight was significantly retarded in both dietary groups among irradiated rats as compared with non-irradiated rats on the same diet. The increment in body weight was slightly less among both irradiated and non-irradiated rats on the 10 per cent protein diet than among those rates on the 25 per cent protein ration. No deaths occurred in any of the groups during the experimental period, nor were any abnormalities observed grossly in their appearance. Histologically, changes were produced in the animals on a protein-deficient diet and not irradiated, which corresponded to changes previously described by others. There was an increase in the size of marrow spaces, with a decrease in trabeculae, a decrease in the number of collagen fibers, and disorganization of the collagen fibers in the periodontal membrane. There were areas of food impaction with slight necrosis of the interdental papilla. The anabolic and catabolic activities in the bone were markedly decreased. In the animals receiving a normal protein diet and gamma radiation amounting to 300 roentgens per week, there were slight changes in the periodontium, consisting of an increase in the marrow spaces, and a slowing-down of osteoclastic and osteoblastic activities. There was a change in the character of the marrow spaces, so that changed myeloid elements were present, but this was not markedly changed. In the irradiated animals receiving 10 per cent protein in the diet the changes associated with gamma

radiation were marked. There was a complete absence of osteoclastic and osteoblastic activity. The bone marrow spaces were very large and filled almost completely with blue-staining myeloid elements. The collagen was reduced in all the tissues of the periodontium. There was a decrease in cellularity, and the collagen fibers of the periodontal membrane were arranged in a haphazard fashion. There was a formation of cystlike areas in the attached gingiva, a few of which were seen in the control animals on the 10 per cent protein diet not subjected to gamma radiation. It is felt that proteins will accentuate the damage to animal tissues, especially from the effects of gamma radiation. Dietary protein depletion or protein deprivation results in disorganization of tissues predominantly composed of protein elements, with a decrease in the protective mechanisms.

17. THE ROLE OF MICRO-ORGANISMS IN PERIODONTAL DISEASE.—*Henry W. Scherp, National Institute of Dental Research, Bethesda, Maryland.* An essential role in periodontal disease is generally attributed to the gingival crevicular microbiota, but the pathogenicity of the individual micro-organisms and their mechanisms of action are incompletely determined. In the present investigations, localized infections resulting in persistent caseous abscesses have been produced regularly in rabbits and guinea pigs by intracutaneous inoculations of 6×10^7 cells of pure strains of small oral treponemas, *Borrelia vincentii* or *Borrelia buccalis* (minimal effective dose, 4×10^6). The spirochetes could be cultured from the lesions for at least 4 days and were found microscopically to have invaded the connective tissue adjacent to the abscesses. Similar infections have been produced with oral strains of *Fusobacterium, Selenomonas,* and *Veillonella.* Lesions were produced also by heat-killed spirochetes and by the insoluble portion of spirochetes disrupted by sonic oscillation, though not by the soluble portion. These experimental infections afford useful model systems for study of the pathogenicity of oral micro-organisms alone and in synergism. Alterations of collagen in the gingiva and periodontal membrane are a regular feature of periodontal disease, but lysis of undenatured collagen by the crevicular bacteria has not been demonstrated unequivocally. In the present experiments, 19 of 26 broth cultures of human subgingival scrapings lysed at varying rates collagen fibrils reconstituted from acid-soluble collagen of rabbit skin. Seven strains of oral veillonellae, 6 of fusobacteria, 4 of streptococci, and 2 of dipththeroids failed to digest this substrate, singly and in various combinations. Singly, these organisms also failed to digest collagen altered by ethylene oxide, but, when combined, they digested it extensively. This result substantiates the often postulated synergistic bacterial lysis of collagen in periodontal disease. Endotoxic activity has often been attributed to oral bacteria but never demonstrated convincingly. In the present experiments, tryptic digestion of acetone-dried cells of three strains of oral *Veillonella* and three of oral *Fusobacterium* released soluble endotoxins, as demonstrated by their production of inflammatory lesions with ischemic necrosis on intracutaneous injection into rabbits and by their ability to produce the dermal Schwartzman phenomenon. Tryptic digests of an oral diphtheroid and an oral anaerobic streptococcus, however, exhibited no endotoxic activity.

V. ORAL ENVIRONMENT. SEPTEMBER 9, 1959. MORNING

18. HORMONAL INFLUENCE OF SALIVARY GLANDS.—*William G. Shafer, Indiana University, Indianapolis, Indiana.* Evidence is gradually accumulating that the salivary glands, at least in the experimental animals, are very intimately associated with various endocrine organs and that a functional disturbance of one of these latter organs may induce dramatic alterations in the structure and function of the salivary glands. The Japanese and French workers were pioneers in this area, although, in more recent years, there have been a voluminous number of studies carried out in the United States pertinent to this problem. Studies in this general area—actually, the experimental dental caries–endocrine organ–salivary gland axis—began in our own laboratories over 8 years ago. Our greatest interest has centered around the effect of the thyroid gland on

salivary gland function in attempting to explain the marked changes that occur in experimental dental caries incidence concomitant with thyroid gland dysfunction. There is little doubt in our own minds and in the minds of others close to this problem that the salivary glands, their morphology, physiology, and pathology, are very closely related to the endocrine system, although the nature of this relationship appears to be a very complex one. Definition of this relationship may do much in the future to solve some of the unexplained facets of the dental caries problem and other oral diseases of man.

19. SALIVARY ENZYMES.—*Howard H. Chauncey, Tufts University, Boston, Massachusetts.* The principal sources of the enzymes present in "whole" or "mixed" saliva are micro-organisms, the secretions emanating from the major salivary glands, leukocytes, and desquamated epithelial cells. Of the more than 30 enzymes studied by various investigators, at least 24 are either partially or wholly produced by the organisms which normally inhabit the oral cavity; 12 are present in the pure secretions generated by the salivary glands; 14 have been detected in human leukocytes, while histochemical investigations of the enzyme content of oral tissues have indicated that desquamated epithelial cells contribute significant amounts of only 1 enzyme. Although the parotid and submaxillary glands produce the major portion of the fluid present in the oral cavity, it is thus apparent that the enzyme contents of the "pure" and "mixed" fluids can be quite different. Furthermore, these differences appear to be due, for the most part, to the cellular elements—micro-organisms and leukocytes—present in mixed saliva. The subject of the bacterial production of enzymes is extremely complex, and considerably more work is necessary to determine the exact contribution originating from each of the numerous organisms present. However, certain pilot studies have shown that many of the organisms have highly stable and distinctive esterolytic enzyme patterns. Similar results have been noted with human "mixed" saliva. These findings have indicated that the measurement of whole saliva enzyme titers, when properly utilized, can be an extremely useful tool for determining naturally occurring variations in the metabolism of oral micro-organism and for evaluating the effect of artificially induced substances on the microbial populations of the oral cavity.

20. EXPERIMENTAL CALCULUS FORMATION.—*Irwin D. Mandel, Columbia University, New York, New York.* Calculus formation was studied by placing contoured celluloid strips around the lower anterior teeth of patients known to form calculus rapidly. Interposing the strip between the tooth and its environment allowed calcareous material to be deposited upon the strip instead of on the tooth. The strips with adherent material were removed at intervals of 2–29 days, fixed, and sectioned without prior decalcification. A variety of stains and staining procedures was used, including the Von Kossa reaction, the Schiff reaction, toluidine blue, and mucicarmine. Examination of the strips disclosed a bacterial plaque common to all salivary calculus. The bacteria are predominantly coccoidal until the fifth day, when the filamentous form predominates. Filaments, therefore, do not appear to be essential to anchorage. Part of the attachment may be due to a cuticle formed by bacteria, zoöglial material, or saliva. The onset of calcification is variable from the third to the fourteenth day. With the onset of calcification, there appears to be a partial loss of metachromasia and a marked diminution in the intensity of the Schiff reaction. The calcifying areas are frequently laminated by alternating dark- and light-staining bands, producing a concentric ringlike appearance similar to that of pulp stones and urinary calculi. The structural and histochemical similarities between salivary calculus and other ectopic calcifications may indicate a common basic mechanism of calcification. The carbohydrate-protein complexes present in the matrixes of calcifying tissues may be the common denominator. Some support is added to this hypothesis by the in vitro diminution in calcification of bacteria plaque by hyaluronidase.

21. CRYSTAL CHEMISTRY OF ORAL CALCULUS.—*Duncan McConnell, Ohio State University, Columbus, Ohio.* Discussion centered on inorganic "stones" that form in

the oral cavity and elsewhere, calling particular attention to the narrow range of biochemical conditions under which they form as compared with "mineralogical" substances of related compositions. Failures to obtain in vitro calculus in the presence of certain antibiotics or after sterilization with heat are interpreted to mean that organisms or their products must remain active in order to form calculus. Inasmuch as the biochemical environments of normal and "pathologic" calcification cannot be greatly different, the factors which govern calculus (or bone) formation must be fairly subtle, and it is possible that a knowledge of the composition and genesis of "stones" can reveal important clues concerning the formation of teeth and bones. The presence of an inorganic phase other than carbonate hydroxyapatite in mature bones and teeth has not been demonstrated by straightforward methods, but there is evidence that the Ca:P ratio may be less than 1.67 for immature or avian bones. Since the Ca:P ratios uniformly exceed 1.67 for "mineral" carbonate apatites, the presence of another substance in immature or avian bone is implied. Such a compound (octacalcium phosphate, with Ca/P = 1.33) has been observed in oral calculi by Jensen, apparently as a stable or metastable substance. If octacalcium phosphate formed as a precursor of bone, its hydrolysis and partial carbonation to yield carbonate hydroxyapatite could be controlled by the activity of an enzyme which is capable of influencing the effective concentration of the carbonate ion (carbonic anhydrase?). Such speculations are not inconsistent with Kazakov's hypothesis for the precipitation of phosphatic nodules (carbonate apatites) on the floor of present-day oceans, except that Kazakov calls upon pressure to govern the concentration of the carbonate ion rather than an enzyme.

VI. BONE. SEPTEMBER 9, 1959. AFTERNOON

22. MECHANISM OF NUCLEI FORMATION AND GROWTH OF MINERALIZING TISSUES. —*Albert E. Sobel, Jewish Hospital of Brooklyn, New York.* The theoretical aspects of nucleation as they apply to bone are discussed. Nuclei formation depends on a metastable solution phase (supersaturation) with respect to the initial solid aggregate and is expedited by catalysis. The complexities of nuclei formation are illustrated by examination of theoretical considerations and equations. Studies of in vitro calcification indicate that nuclei formation requires a minimal product of calcium and phosphate ions. This minimum depends on "local factors" in the matrix. Magnesium-linked inhibition of calcification in vitro by strontium, cyanide, iodoacetate, and fluoride ions suggests two sites in the nuclei-forming molecule or a cluster capturing a single site, competitively blocking the binding of calcium and phosphate ions. Studies of mineralization of non-striated collagen fibers and their transformation to the 640 A spaced striated form by calcium or phosphate also suggest two electrostatically bound groups which can be split by either calcium or phosphate ions. Nucleated rachitic sections mineralize in vitro under conditions in which non-nucleated sections do not, i.e., at a lower Ca $\times$ P product; and they strontify in vitro. Non-nucleated sections do not strontify, indicating a higher degree of specificity for nuclei formation than for nuclei growth. Demineralized bone remineralizes following treatment with chondroitin sulfate or at pH 7.6–8.2, provided that these are followed by $CaCl_2$ treatment. The glycolytic cycle and phosphatase are not required in the minimum system for nucleation, although in the complete system they may play a role. The complete system for nucleation probably depends on (*a*) a specific acid-insoluble collagen; (*b*) sulfated mucopolysaccharide or mucoprotein; (*c*) enzyme systems, such as glycolytic or citric acid cycles; (*d*) energy sources, such as ATP and/or UTP; (*e*) an undiscovered system for concentrating calcium as well as phosphate ions. It is proposed that critical sites in the nuclei-forming macromolecule (a specific collagen, activated by a mucopolysaccharide or mucoprotein and calcium ions) capture calcium and phosphate, creating a caged effect, increasing the probability of their interaction to form nuclei or capture a complete cluster of these ions. The active nuclei-forming center is visualized as an appropriately shaped site where the captured cluster of ions becomes a nucleus. The growth of nuclei into bone hydroxyapatite crystals requires a metastable phase (super-

saturation) with respect to growing nuclei and the presence of impurities. The rate of growth is proportional to the diameter of the particles and is related to the surface-to-volume ratio of the crystal. Habit modification is discussed with relation to its influence on the characteristics of bone crystals. Orientation of the crystals with respect to the collagen fibers of the organic matrix implies that the matrix acts as a habit modifier, inducing growth parallel to the fiber and inhibiting growth of the crystal in a transverse direction. The possibility is considered that the final bone crystal consists of a fused alignment of microcrystals. The small size of bone hydroxyapatite crystals (640 A or less), in contrast with the synthetic hydroxyapatite crystals prepared in the laboratory (2×10^7 A or 2.0 mm.), suggests that factors in vivo operate to limit their size. Cessation of crystal growth in a supersaturated solution is discussed, and consideration is given to the possible roles of surface adsorption of inhibiting ions, a mechanical barrier imposed by the collagen fibrils, and decreased renewal of the fluid surrounding the crystal. The inorganic portion of the mineralized tissue is in two phases: a crystalline core of hydroxyapatite—$Ca_{10}(PO_4)_6(OH)_2$—and an adsorbed outer shell which is of an amorphous or gel-like nature, containing anions, such as carbonate, citrate, fluoride, and cations, such as Na, Mg, Pb, and Sr. This does not preclude entry of a few such ions into the lattice. The inorganic composition of bones and teeth depends on the composition of the surrounding fluids (which can be regulated by dietary constituents). For example, the CO_3:PO_4 ratio of minerals in bones and teeth is related to the CO_3:PO_4 ratio of blood serum.

23. HISTOPHYSICAL STUDIES OF BONE METABOLISM.—*Richard C. Greulich, University of California Medical Center, Los Angeles, California.* Our biological information pertaining to the formation of bone was re-examined. Attention was focused on the role of the osteoblast, on the chemical and morphological nature of the osteoid matrix, and on those factors which may influence the formation of collagen fibrils and their subsequent mineralization. The numerous attempts to characterize the process of mineralization (ossification) in terms of models or by the use of isolated in vitro systems are disturbing. Some of the work which Sobel has done with rachitic cartilage slices and especially the recent studies of Glimcher on the calcifiability of reconstituted collagen (*Rev. Mod. Phys.*, **31**:359, 1959), while done with extreme care, bring us very little closer (if any) to an understanding of the harmonious and orderly processes of bone growth and bone metabolism in the intact organism. The specific nature of the osteoblast was reviewed, and studies by electron micrographs, microradiograms, and autoradiograms were used to emphasize some aspects of structural-functional significance in this cell. What is known of the structure and composition of the so-called osteoid matrix was summarized, and it was pointed out that this area or substance is, after all, of the utmost importance in any consideration of bone formation and growth, be it from structural, functional, or chemical points of view. Since it is in the osteoid that one can visualize the earliest stages in the formation of bone collagen, factors which may control the creation of morphologically recognizable and structurally integrated collagen fibers were discussed. Some recent facts which have been obtained in the author's laboratory relating to the abnormal metabolism of sulfomucopolysaccharides and collagen synthesis in scurvy (Kodicek and Loewi, *Proc. Roy. Soc.* [*London*], **144**:100, 1955; Dunphy *et al.*, *Ann. Surg.*, **144**:304, 1956) were introduced. Those factors which may cause mineralization in bone formation with special reference to mucopolysaccharides (Slack, *Am. J. Med.*, **26**:113, 1959) were mentioned.

24. METABOLISM OF THE INORGANIC PORTION OF BONE.—*Felix Bronner, Hospital for Special Surgery and the Margaret M. Caspary Clinic, New York, New York.* Kinetic studies with radioisotopes were reviewed to point out what is and what is not known about the metabolism of the inorganic portion of bone. Difficulties of interpretation were discussed, with special reference to the distinction between ionic exchange and acretion. The following factors involved in calcium metabolism were discussed

briefly: absorption, vitamin D, renal handling of calcium, and parathyroid control. New information was presented to indicate that there is transtubular movement of calcium in the nephron. It is emphasized that the central mechanism of the control of calcium metabolism is as yet unknown, in spite of the many studies dealing with various aspects of calcium metabolism.

SUMMARY.—*Seymour J. Kreshover, National Institute of Dental Research, Bethesda, Maryland.* During the past three days we have experienced a process of intellectual cross-fertilization, which has made it possible to bring to bear on international health problems the thoughts and scientific efforts of a group of outstanding researchers. I am certain that in the course of this conference we have become increasingly aware that effective progress in dental research involves horizons considerably broader than those encompassed in the activities of any single research unit, school, institution, or country. Each day, the shrinking world encompasses a greater community of interests in health and disease problems.

In our efforts to improve dental health in the United States—just as in your own efforts to do the same in Sweden, Switzerland, Germany, the Netherlands, England, Norway, and so around the world—we see all too clearly the barriers which must be crossed, the roadblocks to knowledge which must be swept aside, and the limitations in our own research efforts. Where is there demonstrated better than here today the important contributions being made by scientists of many nations? However, the speed and adequacy of disseminating scientific information, the methods for free exchange of ideas, the opportunity to consult scientists with specialized knowledge and experience, the ways of learning new techniques and procedures—all are of vital importance if we, as research workers, are to gain greater insight into the illusive etiology of such disorders as dental caries and periodontal disease.

Major breakthroughs in research have come from many nations. They come from those rare individuals with originality of thought, observation, and reasoning who may reside anywhere. This talent is the exclusive possession of no one nation or group of people.

Even as illustrated so many times in the course of the past three days, the worldwide distribution of disease, related to natural and man-made environmental conditions, reveals variations which could provide significant insight into the causation and dissemination of dental caries, periodontal disease, and other oral diseases. Such variations and contrasts, studied systematically, may well provide the key clues to the control and eradication of oral diseases. Most certainly, basic knowledge relating to the pathogenesis of caries and periodontal disease, their origin, modification, and distribution, will be achieved only through long-range integrated studies by laboratory and clinical investigators working co-operatively with epidemiologists in countries where climate, diet, and cultural patterns differ from our own and your own.

Permit me to refer very briefly to some high points of the past three days, not losing sight of the parallel contributions to the success of this conference that have been made by the discussers. As a first-act performance in setting a well-designed stage for the use of those to follow, Brudevold, Ericsson, Trautz, and Muhlemann are to be congratulated for offering fruits of labor so carefully measured for quantity and quality as to provide nutrition without colic. I was particularly pleased to note Ericsson's comments on the splitting of salivary mucoid and his taking-off from that point to call attention to the possibility that this process might liberate calcium ions to produce a state of true saturation and conditions generally favorable to the precipitation of calcium salts on enamel surfaces. I say "pleased" because his example and suggested explanation of why a presumably large consumption of sour milk by Scandinavians is not related to increased caries attack bolsters quite nicely our own spirit of optimism related to a clinical evaluation of dibasic calcium phosphate as an effective caries-control measure when added to the dietary of selected population groups. Here, by "our own spirit of optimism," I presume to speak for studies under way by the East-

man Dental Dispensary, as well as the National Institute of Dental Research. The important role that phosphate minerals may play in dental caries also received a most scholarly review by Harris.

Having had the opportunity just about a year ago to visit Muhlemann in his laboratory, I was particularly gratified to be brought up to date on his interesting experimental approach to provide us with a better understanding of the mechanisms of beginning carious lesions on the enamel surface. After pointing out that natural carious lesions of enamel are generally not initiated by a process of cavitation but rather by the formation of so-called white spots which show an apparently unbroken hard surface of normal microscopic relief, Muhlemann showed in his experiments that acid buffers, although causing loss of glossy enamel surface by etching, do not produce white spots, nor do chelating agents, such as EDTA, despite their preferential dissolution of the interprismatic substance. In contrast, acidified gelatin, with a pH of 3.85, although inhibiting removal of phosphorus by about 50 per cent, produced opaque white spots, denoting subsurface demineralization.

In a consideration of nutritional and dietary factors in dental caries, Cremer and Backer-Dirks have done a superior job in emphasizing and illustrating the so-called exogenic and endogenic mechanisms of influence on caries etiology. Perhaps more than at any other point in this three-day conference, these gentlemen have provided an example of how man-made environmental conditions can provide key clues to our better understanding of dental disease. With the further contributions to the conference provided by the fine reports of Harris and Hess, a compact picture of histochemical and nutritional factors was painted, which brought home to us, with considerable impact, the magnitude of the problem still to be faced in learning substantially more about the specific nature of dental caries. However, in the midst of this great complexity, a ray of early-morning brightness begins to appear; and I refer now to the notable contribution of Keyes, which provides evidence that dental caries, at least in the experimental animal, is an infectious and transmissible disease. With this observation, we are now coming to recognize that the cariogenic flora is part of the microbic complex of the alimentary tract and that the acceleration and deceleration of caries can be affected by many factors, both individually and simultaneously.

Referring to that portion of our conference concerned with the oral soft tissues, a considerable fund of information has been given us through the noble efforts of Forscher, Schultz-Haudt, Person, and Cohen. While in no way wishing to single out our friends from across the sea (that is, not intentionally), permit me to comment just briefly on a few more blue-chip assets rung up in our three-day bank of knowledge. Schultz-Haudt, in his paper, has given us an important insight into what may be the true nature and mechanism of tissue destruction leading to periodontal disease. Observing that the apparent breakdown of tissue in chronic inflammation seems to be due to a disorganization of the collagen fibers and a failure of precipitation of the fibers from precursors, Schultz-Haudt reminds us that tissue alterations might actually be secondary to changes in the ground-substance polysaccharides, including the state of aggregation of the polysaccharide-protein complexes and quantitative differences in the synthesis of hyaluronic acid and chondroitin-sulfuric acid. Additional information regarding the pathogenesis of periodontal disease was contributed by Bertram Cohen, who called attention to his observation that the interdental gingival septum in newly erupted teeth is covered not by oral epithelium but rather by enamel epithelium. This would suggest that the septum constitutes an area of maximum vulnerability during the period immediately after tooth eruption, the vulnerability presumably existing until the time that protection of the underlying periodontal fibers is afforded by oral epithelium growing through beneath the contact point of proximating teeth from papilla to papilla.

I wish that there were sufficient time to permit me to comment with appropriate words of commendation and appreciation on the contributions to this international conference made by today's essayists and discussers. Certainly, it has been a profitable

experience to hear, at first hand, some of the significant findings of Shafer and his associates regarding the so-called three-way axis or interrelationship that exists between experimental dental caries, the salivary glands, and the endocrine system. And then, too, in Chauncey's review of salivary enzymes and Mandel's and McConnell's reports of their investigations of calculus, we have much to mull over on the important subject of so-called oral environmental influences on health and disease. The still digesting dessert of this afternoon's bone meal, we will have to admit, was made most palatable by the clear and concise reports of Sobel, Greulich, and Bronner.

Before closing, permit me just one minute more so that I may refer to Dr. Sebelius' performance of last evening. By so vividly bringing to our attention a firsthand account of the way of life, health conditions, and dental problems in many parts of our world, including those generally thought of as remote areas of our global population, we have forcibly brought before us the magnitude of our responsibility to contribute tirelessly and with continued effectiveness to a better understanding of oral health and disease—problems which recognize no barriers of geography.

International Association for Dental Research

*Scientific Proceedings of the British Division**

Compiled by E. D. FARMER, Editor, British Division

1. FRACTURE CHARACTERISTICS IN POLYMETHYL METHACRYLATE.—*D. C. Smith, Turner Dental School, Manchester.* A detailed study of the mid-line fracture of upper acrylic dentures has revealed that the surface of the fracture presents certain characteristics which distinguish it from the fracture of similar dentures outside the mouth. In order to elucidate the actual mode of failure under clinical conditions, a detailed study has been carried out on the fracture characteristics of polymethyl methacrylate in its dentally molded and cast forms. Under most conditions, failure of this polymer occurs essentially in a brittle manner. The fracture surface is found to display characteristics which indicate a fracture mechanism which is dependent on the microinhomogeneity of the material. Figures are formed through the initiation of secondary fractures ahead of the primary crack, which then unite with it to form the completed fracture surface. Centers of such secondary initiations are microcracks, inclusions, or stress-raising discontinuities. In the case of dental polymethyl methacrylate, the grain boundaries between the old and new polymer provide additional localized areas of microinhomogeneity. Intersection of such isolated secondary fractures with the main crack usually produces a parabolic or a hyperbolic marking, depending on the relative velocities of the two cracks. Unnotched specimens usually display alternate rough and smooth zones which indicate a varying over-all crack velocity. Notched specimens show a smoother surface, on which Wällner lines and spectral colors may be distinguished. Using the evidence provided by such characteristics and the fact that shattering and crack multiplication depend on the stored energy in the specimen at the moment of fracture, it is possible to deduce the mode of failure of a service fracture. In this way the mid-line fracture of full upper acrylic dentures has been shown to be a fatigue failure.

2. OBSERVATIONS ON THE REST POSITION OF THE MANDIBLE.—*A. S. T. Franks, Institute of Dental Surgery, University of London.* Two methods are available for recording the rest position: (*a*) direct measurement, (*b*) cephalometry. Because repeated observations were required, radiographic techniques were not used. A control experiment indicated that direct measurement by means of skin marks and calipers was fairly reliable, and this method was adopted for the study. Assuming that at rest there is minimum muscle activity, an attempt was made to use an electromyographic recording as a control, but this was unsuccessful. Recording the activity of the masseter muscle, it was found that slow opening and closing movements through the rest position did not produce visible alterations in the base line. Ten subjects with complete normal dentitions were investigated by a series of daily measurements of the rest position in five different body postures. A significant relationship was found, the mandible tending to approach the maxilla as the subject came nearer to lying horizontal (on his back). Repeated recordings with cobalt-chromium onlays on the lower posterior teeth—worn for (*a*) 1-hour, (*b*) 12-hour periods—showed an increase in the mandibular-maxillary space in each of the body postures when compared with the previous results. Readings were taken on eight subjects before and after complete extractions.

* The British Division held its eighth meeting at the Royal Dental Hospital of the London School of Dental Surgery, on Thursday and Friday, April 7 and 8, 1960. The meeting was attended by 114 members and guests. Forty-two papers and 6 demonstrations were presented.

After extraction, six subjects showed a decrease in the resting facial height. From the results of these experiments, it is suggested that the rest position of the mandible is related, within the limits of the experimental period, to gravity and change in mandibular load.

3. PRELIMINARY OBSERVATIONS ON THE COMPOSITION OF THE NITROGENOUS FRACTION OF DEVELOPING ENAMEL IN HUMAN FETAL TEETH.—*J. E. Eastoe, Department of Dental Science, Royal College of Surgeons, London.* The jaws were removed from an unfixed 33-week human fetus and X-rayed to determine the state of the developing teeth, which were then carefully removed. In the most-developed teeth, a calcified cap consisting of dentin surrounded by partially calcified enamel could be easily separated from the soft tissues. The caps from the two upper central incisors were wiped carefully with cotton wool moistened with a little normal saline. The white crystalline enamel, which was readily fragmented to a powder, was scraped from the more coherent underlying dentin with a dental excavator under the dissecting microscope. Approximately 11.4 mg. of air-dried, partially calcified enamel was obtained from the two teeth. This dissolved completely in 6 N HCl and was hydrolyzed at 100° for 48 hours. A total α-amino nitrogen determination suggested that the enamel contained some 20 per cent of nitrogenous constituents. Amino acids were determined by the semimicro method of Eastoe (*Biochem. J.*, **74**:8P, 1960), using 0.8-mg. loadings on the columns. Some 96 per cent of the amino nitrogen was accounted for as amino acids. The hydrolysate contained a very high proportion of proline, a substantial amount of glutamic acid (probably mainly as amide), and smaller amounts of fifteen other amino acids. Hydroxyproline was absent, showing that separation from dentin was complete. The amino acid composition was entirely different from reported values for proteins from mature enamel, collagen, epidermis, and blood proteins.

4. THE KERATINIZATION OF GINGIVAL EPITHELIUM IN THE MONKEY.—*W. D. McHugh, Dental School, University of St. Andrews, Dundee.* Cell morphology and the distribution of birefringence and sulfydryl and disulfide groups in gingival epithelium were studied and compared with the corresponding features of epidermal epithelium in 15 *Rhesus* and 15 *Cynomolgous* monkeys. The methods of Chèvremont and Frédéric (*Arch. Biol., Paris*, **54**:589, 1943), Bennett (*Anat. Rec.*, **110**:231, 1951), and Barnett and Seligman (*J. Natl. Cancer Inst.*, **14**:769, 1954) were used to demonstrate sulfydryl and disulfide groups. The deeper layers of the epithelium of the attached gingivae and of the epidermis were virtually identical in morphology, birefringence, and -SH and -S-S- distribution. The surface layers of both epithelia were similar, except that, in the epidermis, there was a well-marked stratum granulosum rich in -SH groups and an outermost layer with few -SH and -S-S- groups. It thus seems unlikely that oxidation of -SH groups to -S-S- groups takes place to any great extent in gingival keratinization, and it is suggested that the differences between epidermal and gingival keratin are due to differences in the amount and character of the cementing substance between the keratin chains. The epithelium at the gingival crest and lining the gingival crevice showed no evidence of keratinization. The primary enamel cuticle was rich in both -SH and -S-S- groups but was not birefringent. The secondary enamel cuticle contained very few -SH or -S-S- groups, was not birefringent, and showed none of the other signs of keratinization.

5. HISTOLOGIC FEATURES ASSOCIATED WITH TOOTH ERUPTION.—*B. Cohen, Department of Dental Science, Royal College of Surgeons, Lincolns Inn Fields, London, W.C.2.* A histologic study has been carried out on human material removed at autopsy from subjects between the ages of fifteen and seventeen years in whom third molar teeth were in the immediately pre-eruptive or early posteruptive stage. The appearance of the interdental septum as seen under a dissecting microscope after removal of the enamel by decalcification in acid showed that the oral mucosa is in

continuity from palatal to buccal papilla only in some cases; in others the interdental col appeared to be devoid of this normal covering. These gross appearances were amplified by photomicrographs of the interdental region from five cases chosen to illustrate various stages in the eruption of the third molar. The histologic features were compatible with the suggestion that when a tooth erupts in close contact with an adjacent tooth, a breach in the continuity of the interdental epithelial integument is inevitable.

6. The Vascular Supply to the Tooth in Relation to the Disposition of Its Roots.—*W. A. Gaunt, M.R.C. External Scientific Staff, Royal Holloway College, Englefield Green, Surrey.* The location of the vessels passing through the apical foramen has been plotted during ontogeny in the case of the upper and lower cheek teeth of the mouse. In the early cap stage, the vessels entering the dental papilla have a mean diameter of 6–7 μ. They attain their maximum number during the generation of the primary cusps, after which they diminish to a consistently low number. An increase in their mean diameter to 9–10 μ coincides with a reduction in their number. The variation in number of vessels during ontogeny can be correlated with their increase in diameter and with changes in the volume of the dental papilla. From the early cap stage there is greater vascularity at the anterior end of the first lower cheek tooth than posteriorly, coinciding with the growth of the primary cusps. The vessels soon divide into an anterior and posterior group, with few vessels between, corresponding to the future position of the two roots. It is into the sparsely vascular region that the root diaphragm grows from lingual and buccal sides. In the equivalent upper tooth, the vessels first congregate in the mid-region on the palatal side and toward the posterior end of the crown. A third anterior group of vessels is soon established, and the tongues of the root diaphragm expand between them to delimit the three roots. It would appear, then, that the distribution pattern of the vessels predicts the ultimate siting of the roots.

7. A Histochemical Study of Dentin.—*N. B. B. Symons, Dental School, University of St. Andrews, Dundee.* Serial sections of undecalcified teeth were cut with vulcanite-bonded aluminum oxide disks. The teeth were sound premolars and molars extracted for orthodontic reasons. Sections were stained with toluidine blue, alcian blue, and the P.A.S. method. The thickness of the mounted sections was never more than 45 μ. With the methods used, three regions can be distinguished in the dentin matrix: the predentin, a narrow intermediate zone of dentin immediately adjacent to the predentin and heavily stained compared with it; this heavily stained zone merges with the rest of the dentin, which is lightly stained by the P.A.S. method and virtually unstained with toluidine or alcian blue. The odontoblast processes stain deeply with all stains in the predentin, in the intermediate dentin, and for a little distance into the "chromophobe" dentin. These heavily stained processes are of considerable diameter and have a dense appearance. The peritubular zone is first distinguishable as a typical translucent structure about the junction of the intermediate and "chromophobe" dentin. It is unstained by the P.A.S. method and only superficially by the other stains. Surrounding each odontoblast process in the intermediate dentin there is an area of dentin matrix which stains heavily with toluidine and alcian blue. This area seems to correspond to that of a developing peritubular zone. In the "chromophobe" dentin, only the very periphery of the odontoblast tubule is stained. The staining throughout with toluidine blue is metachromatic.

8. Changes in Dentin Following Cavity Preparation Using Turbine Handpieces.—*I. R. H. Kramer, Institute of Dental Surgery (University of London), Eastman Dental Hospital, London, W.C.1.* In an investigation of the pulp response to cavity preparation using turbine handpieces, cavities were prepared in about 200 non-carious premolars to be extracted for orthodontic reasons. Borden airotors running at approximately 320,000 rpm under a 2–2½-oz. load were used, cutting (1) with

diamond cylinders, (*a*) with water spray, (*b*) with no water spray; and (2) with tungsten carbide cross-cut fissure burs, (a) with water spray, (b) with no water spray. The cavities were filled with zinc oxide and eugenol unless immediate extraction was intended; experiment duration ranged from a few minutes to 231 days. After extraction, paraffin sections were prepared. In addition to pulp changes, it was noted that, in some experiments, changes had occurred in the dentin bordering the cavity. These changes appeared to include varying degrees of damage in the collagenous matrix and were divided into two categories provisionally termed "burning" and "darkening." "Burning" was never seen in control teeth with cavities cut at conventional speeds. The incidence was low when the turbine was used with water spray but reached 60 per cent when the bur was used dry. "Darkening" was occasionally found after cutting by means of conventional handpieces, but after cutting using turbine handpieces without water spray the incidence was 80 per cent. Special stains showed changes in the collagenous matrix, and all the changes have been reproduced in teeth in which areas of dentin were exposed to heat after extraction.

9. The Inheritance of Connate Incisors in the Dog.—*A. D. Hitchin and I. Morris, Dundee Dental School, Queen's College, University of St. Andrews.* Pilot experiments on the inheritance of geminated incisors in the dog have been described (Hitchin and Morris, *J. D. Res.*, **37**:755, 1958). By breed selection in Lakeland terriers, what one now prefers to term "connate" incisors occurred sufficiently frequently to suggest a genetic basis. These experiments have been continued and the following variants observed among 93 dogs bred: (*a*) Bilateral connate primary first and second incisors in the maxilla followed by a large incisor instead of first and second incisors on each side. Mandibular incisors normal (16). (*b*) A condition similar to *a*, occurring unilaterally. Mandibular incisors normal (12). (*c*) Normal primary teeth in the maxilla succeeded on one side by connate maxillary first and second incisors, the other side and mandibular incisors normal (3). (*d*) Normal primary incisors on one side succeeded by connate maxillary secondary first and second incisors, with condition *b* on the opposite side. Mandibular incisors normal (2). (*e*) Bilateral maxillary connate first and second permanent and normal primary incisors. Mandibular incisors normal (1). (*f*) Normal maxillary incisors in both dentitions, but connate primary mandibular first and second incisors followed by a large secondary incisor unilaterally (2). (*g*) Maxillary incisors as in *a* and mandibular incisors as in *f* (1). (*h*) Macrodont primary first maxillary incisor on one side and condition *b* on opposite side. Mandibular incisors normal (1). In only 3 out of 25 litters were all the dogs without connate incisors, and, in at least 40.8 per cent of the dogs bred with this intent, one of the above variants occurred. Connate incisors in the dog are, therefore, inherited, and studies on the ontogeny of the condition continue, in order to elucidate the primary abnormality.

10. Further Measurements of the Casting Shrinkage of Dental Cobalt-Chromium Alloys.—*R. Earnshaw, Turner Dental School, Manchester.* A report has already been made (Earnshaw, *Austral. D. J.*, **3**:159, 1958) of the casting shrinkage of five dental cobalt-chromium alloys. That study has now been extended to include the 20 alloys of this type available to the writer. Measurements of casting shrinkage were made on cast rods 0.25 inch in diameter by about 2.5 inches long, sprued to their centers. Two nickel-rich "low-fusing" alloys showed a casting shrinkage of about 2.05 per cent. The other 18 "high-fusing" alloys had casting shrinkages in the range 2.13–2.33 per cent. It was shown that variations in the carbon content of a given alloy affected its casting shrinkage.

11. Variations in the Composition of Saliva throughout Pregnancy.—*R. L. Speirs, Physiology Department, King's College, London.* Salivary composition may alter during pregnancy as a result of hormonal, dietary, or metabolic changes. Salivary changes might explain the association between pregnancy and gingivitis or the more

questionable one between pregnancy and dental caries. The concentrations of mucin in unstimulated whole saliva and of calcium, phosphate, and total hyaluronidase in wax-stimulated saliva were first determined. Rates of flow were not determined. The experimental group consisted of 42 patients whose saliva was examined at regular intervals from about the eighteenth week of pregnancy until 6 weeks postpartum. The control group was composed of female students. Preliminary studies in controls revealed (1) marked variations in salivary composition in some subjects throughout the day, (2) a relationship in some subjects between volume of saliva collected and phosphate concentration, and (3) large daily variations only in hyaluronidase activity. These findings in controls largely determined the experimental approach and the degree of significance which could be attached to the experimental results. There was no consistent variation in the concentrations of salivary calcium, phosphate, mucin, or hyaluronidase throughout pregnancy, nor was there a correlation between the presence of gingivitis and any particular salivary factor. In 8 patients, progressive reductions in the calcium and phosphate concentrations occurred during pregnancy. A marked progressive reduction in the mucin concentration was noted in a few patients. In many subjects an abrupt rise occurred 1–7 days postpartum; this was maintained in a few cases.

12. The Association of Citric Acid with Peptides in Mineralized Tissues.—*A. G. Leaver and R. L. Hartles, School of Dental Surgery, University of Liverpool.* The isolation from human dentin of a complex containing citric acid and a peptide has been described (Hartles and Leaver, *Arch. Oral Biol.*, **1**:24, 1960). Subsequent work has indicated that other mineralized tissues, including human enamel and ox bone, also contain similar complexes. When dentin is treated with N HCl, the complex goes into solution, but when the minerals are reprecipitated by addition of ammonia, it becomes associated again with the mineral fraction. When isolated from acid solution, it is soluble in water, ethanol, acetone, and methyl ethyl ketone. The ammonium and sodium salts are water-soluble but insoluble in organic solvents, while the calcium and barium salts are insoluble in water. It appears that the complex contains only 3–4 atoms of nitrogen for each molecule of citric acid and that it exhibits very few terminal amino acid groups. Analysis of the hydrolyzed peptide (Leaver, Eastoe, and Hartles, *Arch. Oral Biol.*, **2**:120, 1960) has shown it to be highly basic, being rich in arginine and ammonia but with appreciable quantities of valine, leucine, and aspartic acid. Because of the high proportion of citric acid, however, the complex is acid in reaction, forming salts in vitro and presumably being linked to the mineral phase in vivo. Further experimental evidence has indicated that some 30 per cent of the total citric acid content is apparently unassociated with the peptide complex.

13. The in Vitro Reaction of Sound Dentin with Glucose and Glucose Derivatives.—*W. G. Armstrong, Institute of Dental Surgery, University of London.* The presence of carbohydrate in carious dentin has been reported previously (Armstrong, *J. D. Res.*, **38**:1217, 1959; Armstrong, *Arch. Oral Biol.*, in press). The possibility that reaction of the dentin with carbohydrate material during caries might account for characteristic properties of carious dentin is under investigation. It has been found that glucose (GL) and glucosamine (GN), the fermentation intermediates —glyceraldehyde (GA) and dihydroxyacetone (DHA)—and the carbohydrate degradation products—methylfurfuraldehyde (MF) and 5-hydroxymethylfurfuraldehyde (HMF)—can react in vitro with sound dentin (SD) to form products with properties similar to carious dentin. Each product showed a brown coloration, similar to carious dentin, with the following order of intensity: $SD_{GN} > SD_{HMF} > SD_{MF} > SD_{GL} > SD_{GA} > SD_{DHA}$. Each product also showed a marked resistance to collagenase attack, in the following order: $SD_{GL} > SD_{GA} > SD_{DHA} > SD_{HMF} > SD_{MF} > SD_{GN}$. Reaction with fluorodinitrobenzene (FDNB) was used to detect possible destruction or blocking of ε-NH_2 groups, and the most marked effect was observed in the dentin glu-

cose product (SD_{GL}), where a 70 per cent reduction in FDNB-binding capacity was found. Analysis of the basic amino acid content of acid hydrolysates of the various products showed that the greatest loss of lysine and arginine was in the SD_{GL} hydrolysate. Similar, though less marked, effects were observed in the SD_{GA}, SD_{DHA}, and SD_{HMF} products. The results obtained indicate that some characteristic properties of carious dentin could be explained in terms of reaction(s) of the dentin matrix with carbohydrate material.

14. An Apparent Anomaly Relating to pH at Tooth Surfaces.—*S. A. Leach, Medical Research Council, Turner Dental School, University of Manchester.* Because pH is a measure of the amount of hydrogen ions available in a solution at any one instance, a low pH at a tooth surface has often been used to imply that decalcification has taken, is taking, or will take place. That this implication is not wholly justified may be shown by the results of the two experiments. First, if a series of buffers of a weak acid are made up at the same initial pH but of different buffering capacity and a fixed volume of each is allowed to react with a fixed amount of powdered tooth mineral, the greatest amount of mineral will dissolve in the solution of the strongest buffering capacity, and the pH rise will be the least. However, if a given volume of buffer of fixed strength and at the same initial pH is allowed to react with different amounts of powdered tooth mineral, the solubility of the mineral and the final pH will be highest in the solution initially in contact with the most mineral. Equilibrium had been reached in all cases, and excess mineral was always present at the termination of the experiments. In the first instance the amount of mineral dissolved is inversely proportional to the final pH, and in the second instance the amount of mineral dissolved is directly proportional to the final pH. As these two sets of results are diametrically opposed, it is not possible to differentiate between the relative contributions made by each of these two systems in measuring the pH at a tooth surface.

15. The Grinding of Thin Sections of Dental Enamel.—*J. H. Fremlin, Mrs. J. Mathieson, and J. L. Hardwick, Departments of Physics and Dental Surgery, University of Birmingham.* The main difficulty in grinding a thin section of enamel arises from its combination of hardness and brittleness. This difficulty can be overcome in principle if the enamel can be firmly fixed to a rigid slab of material of the same hardness. This has been sufficiently well done by cementing thick, well-polished sections to clean microscope slides with the help of Kodak Adhesive No. 910. The sections are then ground down by hand on flat pieces of plate glass. Successively finer grades of aloxite suspended in oil are used to reduce the section thickness to between 10 and 7 μ. A further reduction of a few microns and a good surface polish are obtained by using a succession of grades of diamond dust on microcloth, followed by a firm polish on pitch, using $\frac{1}{4}$-μ diamond powder dispersed in water. Ground sections of teeth with enamel of 6–8-μ thickness can be produced, but below 4 μ the enamel tends to break up into irregular "islands," 20–25 μ across. Dentin can be ground down to a thickness of a micron or less. The adhesive is homogeneous and transparent (with a refractive index of 1.49), so that the section may be examined directly without removal of its support.

16. The Mineralization of Dentinal Tubules in Vitro.—*G. C. Blake, Institute of Dental Surgery (University of London), Eastman Dental Hospital, London, W.C.1.* Sections 70–80 μ in thickness were cut transversely across the crowns of sound, unfixed, undecalcified teeth, between the occlusal surface and the pulp, so that dentinal tubules were cut transversely over one area. The sections were mounted between neoprene washers and held in an apparatus made of nylon. They were then perfused under pressure for 7 days at room temperature with a "calcifying solution" containing sodium, calcium, potassium, chloride, bicarbonate, and phosphate ions at pH 7.3 (Sobel, *Ann. N.Y. Acad. Sci.*, **60**:713, Art. 5, 1955). The sections were radiographed before and after the perfusion, using the Philips microradiographic apparatus. Micro-

radiographs taken after the completion of the experiment showed that a preponderance of the dentinal tubules had become completely occluded with a relatively dense radio-opaque material, while some of the tubules apparently remained unaffected. This in vitro mineralization of the tubules was of approximately the same degree as that of the peritubular zone normally present. The appearance is similar to that seen in sclerosed dentin associated with lamellae, attrition, and caries and in the roots of teeth as a result of aging.

17. THE NATURE AND ORIGIN OF THE ENAMEL MATRIX.—*F. G. E. Pautard, Department of Biomolecular Structure, University of Leeds.* The enamel matrix contains a small quantity of cystine and is described as a "eukeratin," in spite of a proportion of hydroxyproline large enough to suggest that some collagen might be present. Crystallographic verification of the nature of the proteins in the intact enamel matrix is hampered, however, by the fragility of the decalcified tissue. Recently, the well-preserved matrix of an unerupted decalcified molar tooth has become available (Pautard and Weatherell, unpublished results), and X-ray diffraction studies have suggested that, whereas the diagram from the dentin matrix indicates collagen oriented parallel to the tubules, *no* reflections from collagen can be obtained from the enamel matrix. Instead, another pattern—the cross-β configuration closely associated with supercontracted protein—has been observed with the fiber axis oriented parallel to the axis of the prism sheath. A similar pattern has been found in X-ray diffraction photographs of thin films of trout sperm flagella oriented by stretching. Our other studies on the circumstances of deposition of hydroxyapatite on the flagella of unicellular algae have shown that, in certain conditions, rafts of flagella imbedded in a sheet of calcium phosphate are formed by thriving cells on the surface of culture solutions. The X-ray diffraction and electron-microscope evidence from these investigations is illustrated and correlated, and the possibility is discussed that enamel may be of ciliary origin.

18. THE ELECTRON MICROSCOPY OF AMELOGENESIS IN THE RAT.—*R. W. Fearnhead, London Hospital Medical College.* Several authorities have expressed the opinion that amelogenesis in rodents is an intracellular process. This concept is based on the absence of a cell membrane between the cytoplasm of the formative end of the ameloblast and the newly formed enamel matrix in their preparations. The present study provides evidence which demonstrates the existence of a cell membrane in this situation. The discrepancy between the observations appears to be due to the differences between the effect of araldite and methacrylate imbedding media on the tissue. Developing enamel from rat incisors and molars was imbedded in both araldite and methacrylate after fixation at room temperature (20° C.) in Dalton's fixative or buffered (pH 7.2) 1 per cent osmium tetroxide solution. The electron micrographs which were obtained from sections through the forming front of the enamel show a granular precursor substance which appears to be discharged from the formative end of the ameloblasts into the extra-cellular environment. The extra-cellular granules then undergo fibrillogenesis.

19. THE APPLICATION OF THE X-RAY SCANNING MICROANALYZER TO DENTAL TISSUES.—*A. Boyde, V. R. Switsur, and R. W. Fearnhead, London Hospital Medical College and Cavendish Laboratory, Cambridge.* The X-ray scanning microanalyzer developed by Duncomb and Cosslett (*X-Ray Microscopy and Microradiography* [New York: Acad. Press, Inc., 1957]) utilizes the property of X-ray emission which results from the bombardment of elements by an electron beam. The instrument provides qualitative and quantitative data which can be related to the topography of the surface of the specimen. Thus it is possible to identify an element and to obtain an accurate estimation of the quantity of this element in the surface of the sample. The present instrument is capable of identifying elements having atomic numbers greater than 20. Polished cut surfaces of human and rodent teeth were examined with the microanalyzer. A clear identification and an estimate of 7.6 per cent of Fe in the enamel surface of a

rat maxillary incisor were obtained. In the case of human dentin, the analysis showed more Ca in the peritubular dentin than the intertubular dentin.

20. A Review of Current Studies in the U.S.A. concerning the Relation of Micro-organisms to Certain Dental Diseases.—*Morrison Rogosa, National Institute of Dental Research, Bethesda, Maryland, U.S.A.* This presentation is a general review of recent studies at the N.I.D.R. which may significantly clarify our perspectives and stimulate fruitful research of dental caries, periodontal disease, and calculus formation. It is established that caries is absent in the germ-free animal, that therefore micro-organisms are indispensable in caries etiology, and that it can be initiated by monoinfections of previously germ-free rats with acidogenic organisms (Orland, *J. D. Res.*, **33**:147, 1954; Fitzgerald, personal communication). Also Keyes (*J. D. Res.*, **38**:684, 1959) and Keyes and Fitzgerald (personal communications) have shown that caries susceptibility in hamsters can be altered significantly by inoculation of fecal material or suitable organisms from susceptibles to other animals of the same strain. However, the role of nonacidogenic organisms or a mixed flora in germ-free or conventional animals is still largely unknown. In contrast, periodontal disease and calculus formation have occurred in germ-free mice (Baer and Newton, *J. D. Res.*, **38**:1238, 1959) and calculus in germ-free rats (Fitzgerald, personal communication). Thus micro-organisms are indispensable in the etiology of caries but not in periodontal disease and calculus formation. But probably the latter two are exacerbated by the physical presence of organisms as well as their enhancing enzymes. These and related studies of the oral microbiota involving isolation methods, identification procedures, and metabolic relations are engaging the serious attention of the N.I.D.R. and other investigators in the U.S.A.

21. Phosphatase in Micro-organisms Cultured from Carious Dentin and Calculus.—*W. H. Bowen, Department of Dental Science, Royal College of Surgeons, London, W.C.2.* Small pieces of carious dentin and calculus were crushed in 1 ml. sterile 0.85 per cent saline. Of the resulting suspension, 0.1 ml. was used to inoculate 250-ml. flasks containing 100 ml. of sterile brain-heart infusion–thioglycollate-yeast extract broth at pH 7.2. The flasks were incubated aerobically for 48 hours at 37° C. The organisms were harvested by centrifugation and resuspended in sterile normal saline to give a constant reading in an Eel colorimeter. Both acid and alkaline phosphatase activity, with peaks at pH 5.5 and 8.5, were demonstrated by using *p*-nitrophenyl phosphate as substrate, maleic acid and glycine buffers, and 1 ml. of the saline cell suspension as enzyme source. Sodium fluoride in concentrations of 10^{-1} and 10^{-4} M inhibited both the acid and the alkaline phosphatase. Lesser concentrations had no effect. There was no difference in the enzyme activity of the organisms cultured from calculus or caries. These results tend to suggest (*a*) that bacterial phosphatase may have little importance in calculus formation and (*b*) that sodium fluoride reduces caries activity more by reducing enamel solubility than by acting as an enzyme inhibitor.

22. Manometric Measurement of Acid and Gas Production by the Salivary Flora.—*Marie R. Wasdell, School of Dental Surgery, University of Liverpool.* The oral flora includes organisms which in pure culture produce acid or acid and gas when they metabolize certain sugars; in addition, it contains anaerobic organisms which may produce gas even in the absence of added carbohydrate. Thus it is possible that the pressure changes in a Warburg manometer during the anaerobic metabolism of a given saliva in a bicarbonate buffer may be due to the production of either acid (thereby releasing CO_2) or gas or both. Similarly, in estimating the stimulation of salivary glycolysis by the addition of various compounds, it is necessary to differentiate pressure changes due to acid from those due directly to gas production. These problems have been investigated by a manometric procedure, in which, using a series of flasks, the total gas production and change in bicarbonate concentration (hence acid production) have

been measured at 15- or 30-minute intervals. When wax-stimulated resting saliva was incubated under anaerobic conditions without the addition of substrate, the end product of this endogenous metabolism was almost entirely gas and not acid; upon adding glucose, the major end product was acid, but there was also a small increase in gas production.

23. Nasal Carriage of Coagulase-Positive Staphylococci.—*Marjorie Wedgwood, Department of Oral Anatomy, University of Sheffield.* The emergence of antibiotic-resistant strains of pathogenic organisms now constitutes a therapeutic problem, and this study was made to assess its importance in dentistry, by comparing the nasal carriage of coagulase-positive staphylococci in a dental hospital with that in a general hospital. The 236 subjects included professional and lay staff, clinical and preclinical students. Nasal swabs, all taken during the month of January, were cultured on a fibrinogen-agar medium, as described by Klemperer and Haughton (*J. Clin. Pathol.*, **10**:96, 1957), and coagulase-positive staphylococci isolated were tested for varying degrees of penicillin sensitivity. The carriage rates of pathogenic staphylococci, regardless of sensitivity, were higher in clinical groups than in preclinical groups, but the difference was not highly significant (dental subjects $P = 0.3$; medical subjects, $0.05 > P > 0.02$). The carriage rate of penicillin-resistant staphylococci was significantly greater in the dental group than in the medical group ($P < 0.001$) among both clinical and preclinical subjects. The source of contamination in the dental hospital are considered, especially in relation to the emphasis given to aseptic techniques in different departments, and it is concluded that direct contact plays a greater part than nasal carriage in the dissemination of pathogenic staphylococci in dental practice.

24. Some Experiments on the Production of Bacterial Plaques on Teeth in Vitro.—*S. L. Rowles and D. A. Sidaway, School of Dental Surgery, University of Birmingham.* A modification of the apparatus used by Pigman and collaborators (*J. D. Res.*, **36**:314, 1957) is described in which the enamel surface of one tooth, or two teeth in contact, can be exposed to a very slow drip (approx. 0.5 ml. per hour) of one or more fluids, such as nutrient media or Seitz-filtered saliva. The design of the apparatus allows periodic removal of the teeth for examination by bacteriologic, photographic, and radiographic techniques, with accurate relocation of position in the apparatus. Preliminary studies have been made on the survival of micro-organisms in plaques induced by the inoculation of sterilized teeth with whole saliva, using nutrient media alone, or nutrient medium and Seitz-filtered saliva.

25. X-Ray Diffraction Studies of Human Enamel.—*D. F. G. Poole and A. W. Brooks, Dental School, University of Bristol.* X-ray diffraction studies are being made of both permanent and deciduous enamel, using a Phillips flat-plate camera. Results so far show that in permanent enamel there is no true double crystallite orientation. There is normally a diffuse crystallite orientation, which may range over as much as 40°–45°. Within this range, one predominant crystallite orientation is usually found, indicated in the X-ray pattern by an intensification of reflection over a narrow region of the diffuse arc. The angle between the predominant crystallite axis and the prism axis increases from 0° at the cusp to 45° near the cervical margin. Furthermore, over the cusp the orientation of the crystallites occurs within a short concise arc, becoming more diffuse toward the cervical margin. In deciduous enamel "double" orientation was found when taking diffraction pictures of the outer third of the enamel. But, by cutting the collimator size from 0.5 to 0.2 mm. diameter, it was possible to obtain a single high-angle deviation when only the surface zone was examined, a low-angle deviation when the inner enamel was viewed, and double orientation when the junction of the two zones was taken. Looking at sections of deciduous teeth by polarized light showed a zone of crystallites at the surface with a high deviation in 75 per cent of teeth examined. Results from polarized light studies agree closely with those obtained by X-ray diffraction, so that, by examining any section in polarized light, it is possible

to predict the pattern of the arc which would be produced by X-ray diffraction at any given point in the enamel.

26. CRYSTALLINE CONSTITUENTS OF ORAL CALCULUS.—*S. L. Rowles, M.R.C. External Staff, School of Dental Surgery, University of Birmingham.* The results of X-ray diffraction analyses of dental and salivary calculus by the Guinier technique (de Wolff, *Acta Cryst.*, **1**:207, 1948) are discussed. In dental calculus from subjects in the Birmingham area the principal crystalline components were hydroxyapatite, magnesian whitlockite, and octocalcium phosphate. Brushite was detected only infrequently. The order of average abundance was hydroxyapatite>whitlockite>octocalcium phosphate>brushite. Supragingival calculus contained, on average, more octocalcium phosphate and less whitlockite than did subgingival calculus. In salivary calculus, whitlockite and hydroxyapatite were the principal components. Brushite and octocalcium phosphate were seldom found. Monetite, calcite, and calcium oxalate monohydrate, whose presence as minor constituents in oral calculi has been reported, have never been detected in clinical samples of oral calculus. In a small number of samples the diffraction patterns show single additional lines due to phases so far unidentified.

27. MICRORADIOGRAPHY AND INTERFERENCE MICROSCOPY OF CELL TYPES IN RAT SALIVARY GLANDS.—*J. P. Waterhouse, London Hospital Medical College.* Two independent methods of measuring dry-mass distribution at a cellular level have been applied to rat submandibular salivary glands as an introduction to the study of the cellular physiology of these glands. Sections were cut from unfixed fresh tissue with a freezing microtome and from 10 per cent NFS-fixed paraffin-embedded tissue. This material was obtained from Chester Beatty albino rats. A Baker interference microscope with a half-shade eyepiece was used to determine the optical path difference between fresh sections and isotonic saline or an isotonic solution of bovine plasma albumin, and between fixed sections and xylene or terpineol, thus obtaining two sets of values. Microradiographs of fresh and dewaxed paraffin sections and of an adjacent cellulose nitrate step wedge were recorded on cut squares of Kodak M.R. scientific plate, with ultrasoft X-rays having a peak intensity at 8–16 A. Each square was then developed and a photomicrograph of the image of wedge and section examined, using a double-beam microdensitometer. Microradiographic and microinterferometric readings of acinar and granular duct cells in pairs close to each other showed a consistently higher dry mass per unit area for granular duct cells, the approximate ratio being 2:1, for both fresh and fixed material.

28. THE UPTAKE OF S^{35} BY CARTILAGE.—*J. A. Weatherell and S. M. Weidmann, Biological Research Unit, School of Dentistry, University of Leeds.* Investigations into the uptake of S^{35} by calcifying and non-calcifying cartilage were carried out in young rabbits, cats, and dogs. The injected tracer entered the areas of hypertrophying cartilage, which were about to calcify, much more rapidly than in noncalcifying hyaline regions. When slices of epiphyseal cartilage were incubated in Krebs-Ringer phosphate solution to which S^{35} was added, the tracer was taken up most rapidly by the same hypertrophic regions of epiphysis as in the in vivo experiments. The uptake of S^{35} in the hypertrophic regions of epiphyseal cartilage slices was significantly reduced by anaerobic conditions and by the addition of DNP to the incubating medium. Since both factors affect oxidative phosphorylation, the above experiment is further evidence for the presence of oxidative enzyme systems in hypertrophic calcifying cartilage (Whitehead and Weidman, *Biochem. J.*, **72**:667, 1959). It also indicates that the ATP, necessary for S^{35}-fixation in cartilage (D'Abramo and Lipmann, *Biochem. Biophys. Acta*, **25**:211, 1957), is largely produced by oxidative phosphorylation. To obtain further information about the relationship between S^{35} uptake and the calcifying front, time-progress experiments were carried out to establish the relationship between sulfated mucopolysaccharide formation and mineralization.

29. A Nutritional Influence on Tooth Size and Morphology.—*P. J. Holloway, J. H. Shaw, and E. A. Sweeney, Harvard School of Dental Medicine, Boston, Mass.* Two-month-old female rats were littermate-distributed between three experimental groups. All received similar synthetic diets except for differences in the carbohydrate-protein content. Group A received a diet containing 8 per cent casein and 83 per cent sugar; group B, one containing 24 per cent casein and 67 per cent sugar; and group C, one containing 72 per cent casein and 19 per cent sugar. After 1 month on this regimen, they were mated with sibling males and then continued on the same diets through pregnancy and lactation. After weaning, the litters were continued on the same rations as their mothers until all their teeth had erupted, when they were killed. The third molar teeth of the rats in group A erupted approximately 7 days after those of groups B and C. The occlusal surfaces of the molar teeth were photographed, and measurements were made both mesiodistally and buccolingually across the negative images. Twelve measurements were made on 20 rats from each group. The mean of every measurement in group A was significantly smaller ($P<0.01$) than its equivalent in either group B or group C. These differences were least in the first molars (3.0 per cent) and greatest in the third molars (15 per cent). No significant differences were noted between groups B and C. In the third molars of group A, the anterobuccal cusps were smaller than in the other groups and in some cases absent. Ten lower second molar teeth from groups A and B were ground to the mid-line and photographed. Measurements were made from the amelocementinal junction on the mesial aspect to that on the distal side. Again the difference between the mean of the two groups (8 per cent) was significant ($P<0.01$). The animals in group A were considerably smaller than those in groups B and C, but no correlation could be found between the percentage differences in tooth size and body weight at any time during the experiment. This is clear evidence that tooth size can be influenced by environment during development, but, because of the possible role of inanition, these results cannot necessarily be interpreted as a specific influence of protein deficiency.

30. Variations in Crown Pattern of the First Upper Milk-Molar of Man.—*P. M. Butler, Royal Holloway College, Englefield Green, Surrey.* Tooth caps from 28 newborn infants, previously used by M. V. Stack to study growth (*J. D. Res.*, **36**:812, 1957), were examined for variations. Specimens ranged from dentin caps with practically no enamel to those in which the crown was nearly complete. Most features of the pattern are represented on the dentin cap, but deposition of enamel modifies the pattern by reducing the distinctness of minor cusps and emphasizing some of the ridges in the central basin of the crown. The pattern shows considerable variability in detail, especially in the distal part of the buccal crest and in the region of the mesial and distal foveae. Variations of 12 minor elements of the pattern that were studied in detail appeared to be independent of each other, but in 9 cases the right and left teeth of the same individual were very similar. The number of factors controlling the pattern must be large, and the observed variability probably implies a genetic diversity in the population from which the sample was taken.

31. Can Fluoride Influence the Occlusion of the Teeth?—*D. P. Walther, Royal Dental Hospital, London, W.C.2.* The examination of 1,000 East Anglian Secondary Modern school children included an assessment of the dental-base relationship, size of Frankfort-mandibular plane angles, occlusions, muscle morphology and behavior, habits, and early loss of deciduous teeth. The sample consisted of 492 boys and 508 girls between the ages of eleven and thirteen years. Examinations were conducted in Colchester, having an adequate to high fluoride content in its water supply between 1.2 and 2 parts per million (153 boys and 159 girls); in Chelmsford, with variable concentrations of fluoride occurring intermittently between 0 and 1 parts per million (160 boys and 181 girls); and in Norwich, deficient in fluoride, only 0.17 parts per million (179 boys and 168 girls). It was found that the incidence of malocclusion was significantly less in the Colchester group; for example, 53.8 per cent of Colchester

children had a Class I occlusion, as against 44 per cent for the total group. It was also found that the percentage distribution of cases with a normal dental-base relationship, normal Frankfort-mandibular plane angle, and normal muscle morphology and behavior were higher in this group. The incidence of definite Skeletal II and definite Skeletal III dental-base relationships did not vary significantly from group to group. The incidence of early loss of deciduous teeth was also analyzed and appears to play only a very minimal part in the etiology of malocclusion, except as regards buccal crowding, overbite, and axial inclination of incisors. This change in the percentage distribution of malocclusion may be accounted for by the difference between ethnic groups, or it might be due to the difference in fluoride content of the water supply. Investigations are proceeding to try to throw more light on the subject.

32. Noise of Air-Turbine Handpieces in Relation to Acoustic Trauma.—*G. A. Morrant, Institute of Dental Surgery (University of London), Eastman Dental Hospital, London, W.C.1.* A preliminary investigation was conducted to ascertain whether the noise level of typical air-turbine handpieces was likely to produce deafness in the operator. Borden Airoter handpieces were supported 12 inches from a condenser microphone in a soundproof and sound-absorbing chamber. A magnetic pickup head close to the turbine was connected to a frequency motor to record speed, while an accurate air-pressure gauge was inserted in the air line after the control unit. Speed and sound pressure level (S.P.L.) measured on a Bruel and Kjaer frequency analyzer were recorded for air pressures of from 10 to 45 lb/sq in. At normal working pressure of 30 lbs/sq in, 80 db was the most frequently obtained S.P.L., the maximum being 83 db. Frequency analysis at this pressure showed that the spectral components were mainly related to the fundamental speed of rotation of the turbine in the 4–7-kc range. Here the S.P.L. varied from 75 to 82 db. Damage risk criteria for acoustic trauma are not firmly established, but there is evidence that the sound-pressure levels of these frequencies might approach the border line of safety if the operator were subjected to this noise continuously during the working day for a number of years. However, intermittent exposure obviously reduces the risk. A controlled program of audiometry on users of the air turbine seems desirable.

33. Comparative Polarized-Light Studies on the Enamel of Various Vertebrates.—*D. F. G. Poole, Dental School, University of Bristol.* When sections of the teeth of marsupials, such as the kangaroo and opossum, are mounted in water and examined between crossed polars, the tubular enamel shows features similar to human enamel. It is negatively birefringent with respect to the prism axis, but, on the whole, the strength of the birefringence is lower than that of human enamel, probably because of the presence of the tubules. If such sections are transferred to absolute alcohol for about 15 minutes and re-examined, the birefringence is notably stronger than before and is of opposite sign. This change begins at the surface, spreads inward, and may affect the whole of the outer half of the enamel. On returning the section to water, the process is reversed, and the enamel assumes its original properties. This phenomenon is produced by any organic dehydrating agent, as well as by concentrated salt solutions, and is always reversible. Areas behaving in a similar manner have been found in the enamel of hyrax incisors, in the enamel of crocodiles and monitor lizards, in the ganoin of *Polypterus* scales, and in human deciduous enamel. Except for the latter, all these different types of enamel have previously been described as possessing areas impermeable to liquids. In fact, such areas can take up water and only show anomalous properties after treatment with dehydrating agents. A similarity between the behavior of these different types of enamel and the "positive zone" around a caries lesion is apparent, and possible explanations are being sought.

34. Some Observations on the Early Lesion of Fissure Caries of Enamel.—*K. V. Mortimer and A. I. Darling, Dental School, University of Bristol.* Longitudinal ground sections of the occlusal fissures of clinically sound young premolar teeth were

examined in various media by polarized light and Grenz ray. Some of the teeth had brown-stained fissures; in the rest the fissures were unstained. Two main types of fissures were found: (i) the deep fissure, which frequently showed a constriction at its occlusal end, and (ii) the shallow groove. In the first group, lesions were found in more than 90 per cent of the fissures examined. These lesions were seen, occurring initially, occlusal to the constriction and usually on both sides of the fissure. In no case was the base of the fissure involved until an extensive lesion involving the enamel deep and lateral to the bottom of the fissure was present. The main line of spread of the lesion was along the striae of Retzius, which are parallel to the fissure. The lateral margins of the lesions are therefore quite sharp, and the enamel is involved for only a short distance lateral to the fissure. The points of entry and other typical features of a lesion of interstitial caries (Darling, *Brit. D. J.*, **105**:119, 1958) were demonstrated. Though occurring less frequently, in the second group the lesions followed a similar pattern. Sometimes, however, lesions commencing initially at the bottom of a groove were seen. Enamel caries was found in relation to brown-stained and unstained fissures but was more common in the former.

35. Further Observations on the "Positive Zone" in Caries of the Enamel.—*A. I. Darling and K. V. Mortimer, Dental School, University of Bristol.* In a previous paper (Darling, *Brit. D. J.*, **101**:9, 10, 1956) it has been shown that the dark zone at the margin of the carious lesion in enamel shows positive birefringence in ethyl alcohol and other dehydrating media. It was also shown that, while this zone was not imbibable with many hydrophobic media, it was easily imbibed with watery or hydrophilic media. On this basis an attempt was made to explain the zone as an effect of dehydration. In the same paper it was noted that the zone could also be imbibed with methyl alcohol, and further work has confirmed this. Since methyl alcohol is a powerful dehydrating agent, the positive zone cannot be a result of dehydration alone. Furthermore, when the positive zone has been imbibed with methyl alcohol, even prolonged immersion in ethyl alcohol will not reproduce the positive zone. The positive zone can be produced only around a lesion imbibed with methyl alcohol by evaporating the alcohol out of the section in air. This phenomenon is apparently due to the presence of spaces in the enamel which are accessible to ethyl alcohol, etc. It is now considered that the positive zone contains minute spaces big enough to admit the molecules of water and methyl alcohol, but too small to admit those of ethyl alcohol, etc. It is hoped to produce further evidence to confirm this view. If, as suggested by Darling and Mortimer (*J. D. Res.*, **38**:1226, 1959), the translucent zone reflects the loss of soluble protein and the insoluble protein with its associated crystallites is present even in the body of the lesion at a stage later than the dark or positive zone, then these minute spaces are unlikely to arise in relation to the organic matrix as such. It seems probable that they are a manifestation of the early attack of caries at a stage approximating the final loss of soluble protein or the initiation of attack on the crystallites exposed as a result of this.

36. Dental Caries Experience in Continuous and Non-continuous Residents of a High-Fluoride Area.—*P. M. C. James, Royal Dental Hospital, London.* A number of epidemiologic comparisons have been made between dental caries incidence in high- and low-fluoride areas. The technique of such comparisons has occasionally been criticized on the grounds of possible bias by the examiner. The present investigation studies the caries incidence in eleven- to thirteen-year-old children attending a secondary modern school in a high-fluoride area. Dental examinations were carried out on 434 children, using a standard method of diagnosis and replaceable explorer points. At the time of examination the children's length of residence in the area was unkown. Of the 434 children, 250 were continuous resident, and 194 were transfers from other districts with very varied lengths of residence. Twenty per cent of the continuous residents were caries-free, against 6 per cent of the transfers, and dental caries was significantly lower in the continuous-residents group measured by both

DMF and DMFS rates. The condition of first permanent molars was studied separately: 42.00 and 39.80 per cent of first permanent molars were sound in boys and girls, respectively, from the continuous-residents group, against 19.88 and 23.02 per cent of the transfer children.

37. Effect of Early Eruption of the Permanent Teeth on Caries Rates.—*J. Miller, Turner Dental School, Manchester.* In a recent survey of caries in children, girls were found to have more caries in the permanent teeth than boys—the permanent teeth of girls erupted before those of boys; in the deciduous teeth, boys had more caries than girls, up to age six years. Meredith (*J. D. Res.*, **25**:43, 1946) reported that the deciduous teeth of boys erupted before those of girls. This relationship of the eruption of teeth to caries offers an explanation for the differences in caries incidence between boys and girls. It was also found that the permanent teeth, in both boys and girls, were erupting earlier in 1958 (i.e., nearer to age eleven years) than in 1925, when the second permanent molar was reported to be erupting nearer to age twelve than eleven (Medical Research Council, *Special Rept.*, No. 97, 1925). This earlier eruption of the permanent teeth offers an explanation of recent increases in DMF rates of the permanent teeth of children. Assessments of caries rates related to age without considering the percentage of teeth present may be interpreted inaccurately as an increase in caries activity.

38. A Survey of Gingival Bleeding in Adolescents.—*B. Cohen, Department of Dental Science, Royal College of Surgeons of England, and D. V. Taylor, Army Dental Service, War Office (AMD6), London, S.W.1.* This investigation was undertaken with a view to determining the nature of the earliest clinically recognizable lesion in periodontal disease. For this purpose a clinical and radiologic examination was carried out on over 400 boys attending the Duke of York's Military School, Dover, and the Queen Victoria School for Boys, Dunblane, Perth. The subjects fell within the age range of nine to eighteen years. In determining the state of the periodontal tissues, bite-wing X-ray examination of the check teeth was used to assess changes in alveolar bone; the absence or presence of undue mobility was noted as a guide to the condition of the periodontal fibers; and the existence of bleeding in response to exploring the gingival crevice with a blunt probe was adopted as a criterion for determining the integrity of the epithelial integument. Bleeding was adopted as a criterion of epithelial damage because it is an obvious and unequivocal sign; a distinction was made between the appearance of a minute haemorrhagic spot and the welling-up of a droplet of blood following the gentle introduction of a smooth, blunt probe into the gingival crevice. The present communication deals mainly with the incidence of gingival bleeding. In all, 9,957 teeth were examined. A considerable difference in incidence was found between interdental bleeding and bleeding on buccal/labial and lingual/palatal surfaces. Bleeding in the latter situation occurred in about 8 per cent of the teeth examined, whereas the incidence interdentally was nearly 30 per cent. Free bleeding was also far more commonly found interdentally than elsewhere along the gingival perimeter; and it was far commoner between teeth in contact with one another than it was in those instances where a diastema existed. In only 21.4 per cent of the subjects examined was there no free bleeding whatever, and only 5.7 per cent showed not the slightest bleeding anywhere in the mouth.

39. Reproducibility and Discrimination in Electric Pulp-testing.—*J. M. Mumford, School of Dental Surgery, University of Liverpool.* A Nitram stimulator was used to determine the threshold of excitability of 60 upper anterior teeth with apparently normal pulps. Four tests were made at intervals of several days or more. To reduce bias, earlier results were not on view during later tests. The indifferent electrode was held in the hand. With the dental electrode on the incisal edge, results were more reproducible than when it was on the labial surface. This might be due to variation in

contact area and/or to surface conduction to the periodontium. For normal teeth, threshold readings ranged from less than 1 to 6 microamps, but at the lower readings the meter was not accurate. Since it is generally considered that, in acute pulpitis and hyperemia, responses may be obtained at currents below normal, it is difficult to see how, with this stimulator, discrimination would be possible. On the other hand, when the project was extended to include non-vital pulps, there was no response even at the maximum current. This maximum was 78 microamps in cases of gangrene but as low as 25 microamps when the canal was dry. This lower maximum current could be due to the high electrical resistance of a dry pulp canal and might be of value in differential diagnosis. The Nitram stimulator supposedly gives a square-wave impulse, but on the cathode-ray oscillograph this was found to be true only at maximum output. An induced a.c. was also discovered, so the results, though of clinical value, are not absolute in a scientific sense.

40. A Preliminary Report of a Study of Abscessed Deciduous Molar Teeth.—*G. B. Winter, Royal Dental Hospital, London, W.C.1.* The maximum area of radiologic rarefaction in abscessed deciduous molar teeth with underlying permanent successors is in the interradicular region. It was thought that the passage of substances through aberrant dentinal canals from a necrotic pulp might account for this feature. One hundred and seven cariously exposed or abscessed deciduous molars were extracted and immediately placed in formol-saline solution. Of these teeth, 6 were found to have macroscopic evidence of root resorption, which would not have been evident radiologically but which would have formed an abnormal communication between the pulp chamber and the interradicular region. Two per cent methyl violet dye was introduced into the pulp chambers and canals of the remaining 101 teeth, after they had been cleaned out and sealed off with wax. They were re-examined 48 hours later, and in only 5 teeth was there any further evidence of aberrant dentinal canals. To investigate whether these canals were anatomical or the result of resorption, the teeth were decalcified, imbedded, and serially sectioned. On microscopic examination, Howship's lacunae covered by a thin layer of "osteodentin" was seen on the walls of the canals, which gave evidence in favor of resorption being involved in their formation.

41. Some Aspects of the Lateral Nasal Wall in Dental Radiology.—*G. R. Seward, London Hospital Medical College, London, E.1.* In order to interpret more accurately clinical radiographs of the human upper jaw, complete upper jaws, disarticulated skulls, and sectioned skulls were studied. The skulls to be sectioned were radiographed prior to their being imbedded in methyl methacrylate. The planes of section chosen were made to coincide with the path of the X-rays as directed for periapical and occlusal radiographs. This produced a number of wedge-shaped sections which were transilluminated, so that tracings of their cut surfaces could be made. The disarticulated skulls were reassembled, bone by bone, and radiographed at each stage in the reassembly. In some specimens metallic markers were placed in such a way as to outline bony landmarks, and these preparations were radiographed. With these methods, it has been possible to identify certain bony landmarks in clinical radiographs. A clearer understanding of the shadows representing the structures forming the lateral wall of the nose has also been achieved.

42. Accumulation of Strontium 90 from Fallout in Human Teeth.—*Surgeon Captain (D) W. E. Holgate, R.N. Royal Naval Medical School, Alverstoke.* Dental tissues which have formed since 1953 contain Sr^{90}, and estimations of the amount present in human teeth extracted in the United Kingdom, during early 1959, have been carried out. The choice and availability of teeth for sampling are discussed. In the sample of third molar and premolar teeth selected for this investigation the concentration of Sr^{90} over the entire tooth lay in the range of 0.1–0.4 $\mu\mu$c/gm Ca, and a rough correlation with age is indicated, although the material so far examined is too limited to be biologically significant.

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*Symposium I. Mechanism of Fluoride Action**

Moderator, JOSEPH C. MUHLER, Indiana University, Indianapolis, Indiana

I. SYSTEMIC

S1. PLASMA FLUORIDE LEVELS.—*Leon Singer and W. D. Armstrong, University of Minnesota, Minneapolis, Minnesota.* An efficient homeostatic mechanism for regulation of fluoride concentration in body fluids is indicated by constancy of plasma fluoride in residents of four communities having communal waters which vary between 0.15 (Rochester, Minn.) and 2.5 ppm fluoride (Gettysburg, S.D.); mean plasma fluoride 0.14 ppm ± 0.0050 (S.E.)–0.16 ppm ± 0.0073 (S.E.), respectively. Slight plasma fluoride elevation was observed when the communal water contained 5.4 ppm fluoride (Lake Preston, S.D., mean plasma fluoride 0.25 ppm ± 0.0124 [S.E.]). No correlation was found between length of residence in a community and plasma fluoride concentration. Radioisotope studies in collaboration with C. H. Carlson have indicated that a milligram of inert fluoride tagged with radioactive fluoride 18 given two human subjects did not cause detectable increase in plasma fluoride concentration, largely as a result of rapid equilibration with the skeleton and excretion of a third of the dose in urine within 4 hours. Radiofluoride studies indicate that 70–75 per cent of whole-blood fluoride is in plasma. Plasma values of thirty hospitalized patients with a variety of chronic diseases were not different from healthy individuals of the same community. Slightly elevated values were observed in three cases of demineralizing diseases (hyperparathyroidism and multiple myeloma) and one terminal patient with carcinoma of lung. Animal experiments support the concept that homeostatic regulation of plasma fluoride is efficient. Large variations in skeletal fluoride concentration in rats could not be correlated with plasma fluoride, and no difference in mean plasma fluoride values was found for colonies receiving 0 to 50 ppm fluoride in drinking water. Bovine parathormone did not alter plasma fluoride values, although calcium serum levels were elevated.

S2. REACTIONS BETWEEN FLUORIDE AND HYDROXYLAPATITE.—*Harold C. Hodge, University of Rochester, School of Medicine and Dentistry, Rochester, New York.* High concentrations of fluoride in solution react with hydroxylapatite to produce calcium fluoride (CaF_2). Giesecke and Rathje (1941) showed that a phosphate release characterizes this reaction. A separate phase appears, as pictured by Gerould (1945). Calcium fluoride solubility is considerably greater than that of apatite; thus the surface deposit of this compound left by a topical fluoride treatment in practice rapidly disappears by solution or attrition.

Low concentrations of fluoride in solution react with hydroxylapatite to produce fluorapatite. Although no direct proof can be offered, the circumstantial evidence is convincing. MacIntire (1937) demonstrated a reduction in acid solubility. Rathje (1952) concluded that fluoride forms an isomorphous replacement up to 70 per cent of the hydroxylapatite. The fluorapatite:hydroxylapatite ratio cannot be calculated from the fluoride percentage because "excess F" positions in the crystal can also be

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* This symposium was held Friday evening, March 18, 1960, as part of the general meeting of the International Association for Dental Research in Chicago. These abstracts were received after the deadline for inclusion with the rest of the material from the 1960 General Meeting (*J. D. Res.*, **39**: 649, 1960).

occupied (Hendricks and Hill, 1950). Neuman *et al.* (1950) quantitatively described, according to the mass law, the reaction between solution fluoride and solid hydroxylapatite as a fluoride:hydroxyl exchange. Unpublished data by the author *et al.* showed that the mobilization of deposited fluoride is not accomplished quantitatively by reversing the conditions of deposition.

S3. Mobilization of Fluoride from the Bones and Teeth of the Growing and Mature Rat.—*I. Zipkin, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.*—Two studies comprised the present investigation. In the first, female weanling Holtzman rats received 10 ppm F in the drinking water as NaF for 70 days, and representative rats were sacrificed at the end of the fluoride regimen and at four 70-day postfluoride periods. In the second study, similar rats received 10 ppm F as NaF for 365 days and were sacrificed at the end of the fluoride regimen and at three 70-day postfluoride periods. The bones were placed in 15 categories, and fluoride was analyzed by standard procedures. Fluoride was concentrated to different degrees in various bones of the growing rat, with sternum, vertebra, and scapula showing the greatest concentration. The total fluoride lost from the bones of the growing rat varied considerably. In fact, the ulna + radius, the vertical ribs, and the hands and feet gained in fluoride. The greatest loss, as previously observed by others, was seen in the incisors. The total loss of fluoride from the skeleton of the growing rat averaged 3.4 per cent over a 280-day postfluoride period. In the mature rat, the bones varied in their ability to concentrate fluoride even after 365 days of exposure to 10 ppm F. Again, the sternum, vertebra, and scapula showed the highest concentration of fluoride. The mobilization of total fluoride from the bones of the mature rat was somewhat more uniform than in the case of the growing rat and averaged 10.8 per cent. Again the incisors showed the greatest percentage loss in total fluoride. The data support the conclusion that fluoride turnover is a local process and probably does not proceed by way of the general circulation, since the continuously growing incisors did not incorporate fluoride during eruption.

S4. The Effect of Fluoride on Oral Bacteria.—*G. Neil Jenkins, Medical School, Newcastle-upon-Tyne, England.* Although there is good evidence to support the solubility theory of fluoride action in caries (Jenkins *et al.*, 1952; Dowse and Jenkins, 1957; Isaac *et al.*, 1958), the possibility has to be borne in mind that antibacterial or antienzymatic effects may also make a contribution. Before it is possible to decide on the validity of the antibacterial theory of the effect of fluoride on caries, three pieces of information are required. First, it is essential to know the minimum concentration of fluoride which can inhibit acid production by salivary bacteria. Second, we must have some basis for considering what degree of inhibition is necessary to exert an appreciable effect on caries. Third, the concentration of fluoride in the plaque under various conditions must be known, and, unless the concentrations reach levels which are capable of inhibiting the bacteria, the theory must be rejected. Unfortunately, information on all these points is still uncertain. The first has been investigated most thoroughly; the other two are still subjects of speculation.

S5. Effect of Fluorine on Bacteria and Organic Components of Dentin.—*B. G. Bibby, Eastman Dental Dispensary, Rochester, New York.* Recent work by Jenkins has confirmed Bibby's earlier findings that, while much higher concentration was needed to interfere with growth, fluorine concentrations as low as 2 ppm would inhibit acid formation by mouth bacteria. In addition, it has been shown that like inhibition occurs in saliva, particularly at pH levels important for enamel decalcification. Although freshly deposited fluoride in the tooth surface will inhibit acid production by microsuspensions of lactobacilli for a short time, the duration of this effect is too short to be important in caries prevention. Beyond the inhibition of glycolytic enzymes, there is no definite information regarding antienzymatic effects bearing on dental caries. Theoretically, the fact that fluorine will combine with proteins could

affect the organic structures of the dentin or enamel or the enzymes attacking them, but definite evidence that such effects take place is lacking.

S6. Posteruptive Effects of Fluorides.—*R. M. Grainger, G. Nikiforuk, and K. J. Paynter, University of Toronto, Toronto, Ontario.* An attempt has been made to integrate data from various published sources dealing with the posteruptive uptake of fluorides by dental tissues and dental caries. It is assumed that, to be effective, the fluorides must, first, be present on (or in) the enamel surface; second, the fluorides must be present in a certain minimal concentration, which may be of the order of 1,200 ppm or greater (Brudevold *et al.*, 1956), and, third, the fluoride should be present prior to the beginning of the caries attack and continually thereafter. Epidemiologic evidence suggests that the maximum caries protection is obtained for teeth of individuals who have been consuming fluoridated water as late as the last year or two prior to specific tooth eruption and that the fluoride in the tissue fluid is probably taken up by the unerupted teeth at the enamel surface. On the basis of Brudevold's work (1956) it appears that the surface fluoride concentration of teeth will gradually (possibly over 15 years) increase to what may be an effective cariostatic level, even from normal dietary fluoride sources. For such teeth not developed in a fluoride-endemic area, the posteruptive time (and hence risk of decay) required for the tooth to acquire an effective surface concentration of fluorides may be reduced if fluorides are available in high concentration from drinking water (Klein 1945; Mavrogordato 1951) or if fluorides are applied topically from concentrated solutions (Volker 1939; Bibby 1944; Knutson *et al.*, 1943). The work of Syrrist and Karlsen (1954) suggests that caries protection from topical sodium fluoride decreases with time, probably because of partial removal of the fluoride by washing and attrition (Brudevold *et al.*, 1957), and it has not been established whether topical stannous fluoride applications are longer-lasting. However, the stannous fluoride appears to be more effective than sodium fluoride when used similarly (Howell *et al.*, 1955) and may be more practical economically, since one treatment a year appears to be effective (Gish *et al.*, 1959). Evidence has been put forward by Muhler *et al.* (1960) that stannous fluoride in a dentifrice will significantly reduce dental caries attack, and it has been suggested on theoretical grounds by Cooley (1960) that daily use of a stannous fluoride dentifrice may enhance (or prolong) the effectiveness of topical fluorides by preventing or slowing down the loss of the topical fluorides by washing or attrition.

II. TOPICALS: HISTORICAL DEVELOPMENTS

Joseph F. Volker, *University of Alabama, Birmingham, Alabama.* Almost a century ago, Magitot observed variations in the resistance of teeth to acid decalcification and called attention to the possibility that the more acid-resistant enamel contained "a minute quantity of fluoride of calcium." Subsequent investigations in the late 1800's by Carnot established the ability of calcified tissues to combine with substantial quantities of fluorides from aqueous solutions. These and other researches stimulated the development of fluoride-containing mouth washes, lozenges, tooth powders, and tooth pastes for the prophylaxis of dental caries prior to the turn of the century. Unfortunately, the experimental methods available to the investigators of the period were inadequate for an effective exploration of the interrelationship. Consequently, the possible benefits of topical fluoride were neglected by the researches of the period 1900 through the 1930's. In this interval, however, agricultural and industrial chemists demonstrated (1) that excessive fluorides in the drinking water could be removed by filtration through beds of bone ash and (2) that the solubility of basic calcium phosphates was determined by their fluoride content. Since our laboratory was engaged in a study of the solubility of dental tissues and since we were aware of the literature cited, we proceeded to expose powdered enamel and whole tooth surfaces to dilute fluoride solutions and observe their subsequent solubility. We noted that under these conditions there was a very substantial reduction in enamel and dentin solubility. Recognizing that this reaction had potential value in caries prophylaxis, we urged that topical

fluoride be tested clinically for tooth-decay inhibition. In parallel researches, other investigators were studying the influence of dietary and water fluoride on experimental caries in the rat. Since these fluorides were not ingested until after tooth eruption, there was additional reason for suspecting a topical fluoride effect. A series of experiments eliminated the possibility of pulpal and salivary gland fluoride influences, and the concept received strong support when Sognnaes showed that topical applications of fluoride to isolated tooth surfaces reduced rat tooth decay. Finally, clinical experimentation with children by Bibby established the validity of the hypothesis.

S7. Fluoride Action as Evaluated by the Artificial Mouth.—*Ward Pigman, T. Koulourides, and E. Newbrun, University of Alabama, Birmingham.* The artificial mouth has been used for two types of studies involving the action of fluorides. Dentrifices and agents for topical treatments have been evaluated. Ground surfaces of human enamel were treated for about 2 hours with solutions of stannous fluoride (0.4 and 4 per cent), sodium fluoride (2 per cent), and sodium fluorophosphate (1 per cent). When exposed to the artificial mouth for 8–10 hours, the rate of softening of these teeth was 27, 35, 50, and 35 per cent, respectively, of that of untreated controls, which softened at the rate of 7.4 $\pm$ 1.8 (S.D.) per cent decrease of hardness per hour. The treatments produced a highly significant increase in resistance of the teeth.

Tooth surfaces softened by the action of 0.001 M acetate buffer at pH 5 were shown to be rehardened to the initial values when exposed several times for 60–120 minutes each at 37° to a solution containing calcium (1.5 mM/l) and phosphate ions (Ca/P ratio, 1.67) at pH 7.3 (initial) in the presence of 0.3 mM/l, of fluoride (6 ppm). The rehardened teeth were at least as resistant to softening by the buffer as were the original teeth. Much longer times were required in the absence of fluoride ions, and the acceleration required as little as 1 ppm of fluoride.

A similar "rehardening" solution was incorporated into the medium used in the artificial mouth, and fresh medium was provided twice daily. Teeth exposed to this medium for as long as 5 days showed no appreciable softening in the artificial mouth. Control teeth, under the usual conditions, were softened below the limits of measurement ($<$ 90 KHN).

S8. Electron-microscopic Evidence of Fluoride-Enamel Reaction.—*David B. Scott, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.* To date, essentially all the electron-microscopic investigations of the fluoride-enamel reaction have dealt with the effects of the sodium and stannous salts on the structure and acid solubility of the outer enamel surface. It has been well established that a calcium fluoride layer is formed when the enamel surface is exposed to aqueous sodium fluoride solutions. The effect of such treatment on enamel solubility has been difficult to demonstrate clearly, but there has been evidence of a slight protection against acid etching. In contrast, when surfaces have been exposed similarly to stannous fluoride solutions, equimolar with respect to fluoride, a very marked protection has been noted when the same etching tests have been applied. Closer inspection of the enamel surface after treatment with stannous fluoride solutions suggests that the increase in resistance to acid may be primarily due to the deposition of a protective surface layer. Samples of this surface deposit have been isolated and, through electron and X-ray diffraction, identified as hydrous hydrated stannous oxide. Further studies of such oxide layers have demonstrated that they are very resistant to washing with water, etching with acid, and brushing with water alone but are quite readily damaged by brushing with an abrasive slurry. Along similar lines, when teeth have been immersed in dentifrices containing sodium or stannous fluoride, protection against acid etching was also noted, that provided by the stannous fluoride dentifrice being considerably more pronounced. If the dentifrices were applied by brushing, however, the degrees of protection were markedly reduced.

S9. Reactions of Tin and Fluoride with Enamel.—*William E. Cooley, The Proctor & Gamble Co., Cincinnati, Ohio.* When pieces of clean, sound enamel from

human incisors are brushed with sodium fluoride or tin fluoride solutions, measurable amounts of fluoride or tin can be found on or in the enamel. The initial rate of reaction of fluoride is much faster than the steady rate established after some minutes of treatment. The steady state for the reaction of tin is one of equilibrium, in which the enamel receives no net increase of tin after the initial reaction. Fluoride penetrates into the enamel, whereas most of the tin is on or near the surface as a uniform, tough coating. Presence of the coating inhibits diffusion, slows reactions, and protects the enamel from dissolution through acidic attack. Both the fluoride and the tin reaction rates are dependent on concentration in dilute solutions. In stronger solutions a change of concentration has little effect until about 0.1 M is reached. Thereafter, fluoride reactivity increases with concentration, and tin reactivity is influenced by hydrolytic precipitation of tin onto the enamel in a form different from the coating of true solution–solid reaction products. Large amounts of tin-containing precipitate gather on enamel whenever treatment is prolonged, concentration or pH is high, or readily hydrolyzing salts such as tin chloride are used. Carious enamel reacts with tin fluoride solutions to take up tin at a rate many times greater than the rate for sound enamel. Under true mouth conditions the rate of reaction is slower than in pure solutions, but still much faster for carious enamel than for sound enamel.

S10. Depth of Penetration of Fluoride in Erupted Teeth.—*Finn Brudevold, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston, Massachusetts.* Although F tends to accumulate in the surface layers of enamel and dentin, some penetration of F into the body structure can occur even in fully mineralized teeth. Evidence of F into enamel has been presented in the following types of studies: (1) Analyses of ground-layer samples of enamel of erupted teeth of young and old persons have shown increased F concentrations with age. The increment is most marked in the outermost surface enamel and decreases gradually in underlying portions. (2) Intact enamel picked up significant amounts of radioactive F and a 1 ppm solution after only 5 minutes' exposure. F penetration was suggested, since F was removed only gradually by polishing away the surface enamel with abrasives. (3) In vitro experiments have shown that exposure of intact enamel to concentrations of F similar to those used in topical applications will bring about F deposition and slight penetration of F into the bulk of the enamel. Similar observations were made on etched and ground enamel surfaces. (4) X-ray and electron diffraction studies suggest that low pH silicofluorides may penetrate some distance into enamel before decomposition and deposition of CaF_2 takes place. (5) The F uptake by powdered enamel on which the surface area was known was found to be greater than could be accounted for by only surface deposition. The amounts of F taken up after 1-hour exposure to solutions containing from 40 to 100 ppm of F suggested surface saturation and some slight penetration into the enamel particles. Penetration of F into dentin was suggested from a study of layer samples, ground from teeth of different ages. There was appreciable increase in the concentrations of F in the surface layers with age. This increase extended farther into the body of the dentin than was the case with enamel.

III. SELF-ADMINISTRATION

S11. The Caries Status of Children Who Ingested Sodium Fluoride Tablets.—*Francis A. Arnold, Jr., F. J. McClure, and Carl L. White, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.* Although ingestion of suitable concentrations of fluoride in drinking water has become an accepted procedure for dental caries control, many other avenues have been suggested for adding fluoride to the diet. One of the most seriously considered of these alternatives is the daily use of fluoride tablets. A certain degree of caries prevention has been reported for this method by investigators elsewhere. In the study outlined here the mean numbers of def and DMF teeth of 121 children who ingested a daily fluoride tablet were compared with corresponding data previously reported for children of similar ages using a natural fluoride water or fluoride-adjusted drinking water. DMF and def data for the two

groups were of generally comparable levels. The daily fluoride-tablet regimen did not result in any cosmetically significant fluorosis. The mean fluoride content of deciduous teeth of children ingesting these tablets was found to be significantly higher than that of children in a non-fluoride area. It also appeared that the fluoride content of the deciduous teeth increased proportionately with the length of time the tablets were ingested. On the basis of these results it appears that fluoride tablets may have merit in areas where children do not have access to a fluoridated water supply. However, the fluoride content of the "home" water supply must also be known, suggesting that, as a public health procedure, this regimen could be used effectively only in controlled programs supervised by health authorities. Based on the operational experience gained in this study, it would appear that water fluoridation remains the most practical and safe procedure for caries control in large population groups.

S12. Blood and Urinary Studies Using Fluoride Tablets.—*David Bixler, Indiana University, Indianapolis, Indiana.* Water fluoridation is now considered an acceptable, safe, and desirable public health method of reducing tooth decay. However, that portion of the population which does not consume a communal water supply will not receive these dental benefits. Accordingly, it has been advocated by some groups that a daily fluoride tablet be administered *per os* as a suitable replacement. Little or no information is available concerning either the metabolism or the anticariogenic effectiveness of such tablets. This paper is concerned with a portion of that problem—the metabolism, as evidenced by blood and urine studies, of a commercially available fluoride tablet. Studies in dogs on reagent-grade powdered NaF clearly show that, following the oral administration by stomach tube of either 5 or 10 mg. of fluoride as NaF, blood fluoride levels reach a maximum peak about 20 minutes after administration. This peak represented about a 50 per cent increase over the control level. Urinary fluoride excretion in these same dogs reached a maximum at about 2 hours. When a commercially available NaF tablet was administered by stomach tube to dogs (1 mg. F^-), the blood fluoride level did not reach a maximum peak until 2 hours afterward. Urinary fluoride excretion in human subjects ingesting 1 tablet per day showed a maximum excretion very similar to that observed after ingestion of NaF as a powder. These results indicate that fluoride from a tablet is more slowly absorbed into the blood stream than is fluoride in a powdered form. The over-all metabolic availability of either fluoride, as indicated by the urine studies, seems very comparable. The fact that blood levels remain elevated for such short periods of time raises the question of their value clinically in reducing human dental caries.

S13. Site of Fluoride Absorption.—*George K. Stookey and Daniel B. Crane, Indiana University, Indianapolis, Indiana.* Numerous studies have indirectly described fluoride absorption and the effect of innumerable factors upon the phenomenon, inferring absorption values from metabolism and skeletal retention values. However, very little experimental evidence is available in which the absorption of fluoride has been directy studied and the site or sites of fluoride absorption from the gastrointestinal tract elucidated. One direct approach to this problem has employed isotopically labeled fluoride, F^{18}. These studies (Wallace-Durbin; Perkins; Armstrong) have shown that the isotope rapidly appears in the blood and have suggested that fluoride absorption occurs in the stomach. Moreover, these studies have shown that 75 per cent of an orally administered dose of isotopically labeled fluoride is absorbed within the first hour after administration. Another direct approach has been undertaken in our laboratories, in which the absorption of fluoride was studied from various isolated sections of the gastrointestinal tract. These segments were surgically isolated in intact anesthetized animals, known quantities of fluoride were injected, and the amount of residual fluoride remaining after various time intervals was determined. The results of these studies have clearly shown that fluoride absorption in the rat is not limited to the stomach but may occur throughout the first 35 cm. of the intestine. These data also confirm earlier findings that the rate of fluoride absorption is quite rapid and indicate a greater rate of absorption occurring in the intestine than in the stomach.

Observations on Pathogenic Coliform Bacteria from the Adult Human Mouth

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The purpose of this study was to isolate the pathogenic coliform bacteria presented in adult human mouths. Within this group of bacteria, some *Escherichia coli* serotypes have been known to be the cause of infantile diarrhea disease. Our consideration of the coliform bacteria in this study, however, covered *E. coli* as well as *Aerobacter aerogenes*. It was also within our interest to determine the presence of antigens and study the pathogenecities of the isolated organisms in comparison with some known pathogenic *E. coli* serotypes.

In the past, cases of epidemic and sporadic infantile diarrhea were reported from which no pathogenic organisms such as *Salmonella* or *Shigella* were received. Occasionally, *E. coli* was isolated.[1, 2] Whether *E. coli* could cause such outbreaks was questioned. At first, most workers were hampered by using only biochemical tests for differentiating the *E. coli* isolated from the cases of diarrhea and *E. coli* from normal individuals. Because of misleading results, some creditable works of Goldschmidt in 1933 did not receive attention.[3, 4]

Bray[1, 5] and Bray and Beavan[1, 6] were probably the first to emphasize the fact that certain *E. coli* serotypes were associated with outbreaks of the infantile diarrhea disease. They isolated bacteria from diarrhea patients, which were identified by Kauffman and Dupont as *E. coli* 0111:B4 serotype.[7] Varela, Aguirre, and Carille[8] isolated a bacterium which they named *E. coli-gomez*, which was later proved to be identical with *E. coli* 0111:B4.[9]

Kauffman[10] in 1947 published an antigenic scheme for *E. coli* serotypes. Since then, many investigators[11–16] have been interested in studying the antigens of *E. coli*, particularly those which are known to be associated with infantile diarrhea.

MATERIALS AND METHODS

The micro-organisms used for this study were 22 cultures of coliform bacteria. They were isolated from the mouths of 254 adult college students of both sexes. Among these 22 cultures, 3 were identified as *E. coli* and the other 19 as *Aerobacter aerogenes*. In addition, four known *E. coli* serotypes*—026:B6, 055:B5, 0111:B4, and 0127:B8—were used as controls.

One-half per cent lactose broth was used as mouth-washing fluid, and samples were

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* Obtained through Kansas State Board of Health Laboratories, Topeka, Kansas.

taken in 250-ml. Erlenmeyer flasks. These samples were incubated at 37° C. for 24 hours. Approximately 1 ml. of each incubated sample was streaked on EMB* and MacConky* agar plates. These plates were incubated at 37° C. and were examined for the presence of coliform colonies which resembled either *Escherichia* or *Aerobacter*. Identification of each culture was confirmed by using the following biochemical media: lactose, glucose, sucrose fermentation media, litmus milk, Koser's citrate medium, MR-VP medium and peptone broth for indole test. Table 1 shows the typical reactions of *E. coli* and *A. aerogenes* on some of these media.

The organisms which were identified as *E. coli* and *A. aerogenes* were recultured in nutrient agar slants and the newly grown 24-hour cultures were tested for the presence of antigens of the four known pathogenic *E. coli* serotypes 026:B6, 055:B5, 0111:B4, and 0127:B8.

Saline suspensions of one to two billion bacteria per ml., or a density corresponding to tube No. 3 on the MacFarland scale, were made from each culture. Thus, by applying the slide agglutination technique, about 0.05 ml. of each suspension so prepared was tested for the B antigens of the four known *E. coli* strains with commercially

TABLE 1

TYPICAL REACTION OF *E. coli* AND *A. aerogenes* ON CERTAIN BIOCHEMICAL MEDIA

Organisms	Lactose	Glucose	Koser's Citrate	MR	VP	Indole
E. coli.....	(+)*	(+)	−	+	−	+
A. aerogenes.....	(+)	(+)	+	−	+	−

* (+) Acid and gas production.

prepared antisera.† The suspensions were then heated for 1 hour at 100° C. to dissolve B antigens. They were then cooled to room temperature, and, by applying the same technique and antisera, the presence of O antigens were tested.

The suspensions containing the antigens of the known pathogenic *E. coli* were fed to chicks. One ml. of previously prepared live organisms was given orally to 1-day-old chicks. Five chicks per culture were inoculated, and 20 chicks were held as controls.

The study of pathogenicity by intravenous injection into chicks was also conducted. Of the afore-mentioned cultures, 0.5 ml. of the saline suspensions was injected into 2-week-old chicks, 5 chicks per culture, and 30 chicks were held as controls. A group of 5 chicks was injected with non-antigenic *A. aerogenes*, and another group of 5 chicks was injected with non-antigenic *E. coli*. In addition, the pathogenicity of the four known strains of *E. coli* was also studied in the same manner by being administered to 5 chicks per culture orally and 5 chicks per culture intravenously.

The chicks killed by the infection and also some non-infected chicks were autopsied for the isolation of organisms. The bacteria were identified biochemically and serologically.

The possibility of the adaptation of pathogenicity of non-pathogenic coliform or-

* Difco Laboratories, Detroit, Michigan.

† Lederle Laboratories Division, American Cyanamid Company.

ganisms by acquiring the antigens of the known pathogenic strains was studied. The 19 cultures of *A. aerogenes* isolated from human mouths were grown together with the known strains of pathogenic *E. coli* 026:B6, 055:B5, 0111:B4 and 0127:B8. Equal volumes of inocula of both bacteria were mixed, and nutrient broth was added. After 24 hours of incubation at 37° C., the mixed cultures were streaked out on differential media. Pure cultures were obtained from the colonies which appeared to be neither typical *E. coli* nor typically *A. aerogenes* for the confirmatory study on their biochemical characteristics and antigenicity.

TABLE 2

ORAL ADMINISTRATION OF ANTIGENIC *A. aerogenes* TO 1-DAY-OLD CHICKS

CULTURE NO. OF *A. aerogenes* INJECTED	TESTS								
	After 1 Day			After 5 Days			After 2 Weeks		
	Recovery	Test with 0111: B4 Antiserum		Recovery	Test with 0111: B4 Antiserum		Recovery	Test with 0111: B4 Antiserum	
		O	B		O	B		O	B
A5*	+	−	+	+	−	−	−	−	−
B6*	+	−	+	−	−	−	−	−	−
B11*	+	−	+	−	−	−	−	−	−
D18*	+	−	+	−	−	−	−	−	−
H9*	+	−	+	+	−	+	−	−	−
J15*	+	−	+	−	−	−	−	−	−
E10†	+	−‡	−‡	−	−‡	−‡	−	−‡	−‡

* *A. aerogenes* cultures possess B antigens of *E. coli* 0111 : B4 serotype.
† Control, non-antigenic *A. aerogenes*.
‡ Negative agglutination with all four antisera.
Intestines were considered to be the best source for recovering the organisms administered.

RESULTS

Twenty-two coliform cultures were isolated from 254 mouth-wash samples (8.6 per cent). Among these, 3 were *E. coli* (1.2 per cent), and the rest were *A. aerogenes* (7.4 per cent).

None of the isolated *E. coli* was found to possess the O or B antigens of the known pathogenic serotypes upon slide agglutination test. However, 6 cultures of the isolated *A. aerogenes* were found to possess the thermolabile envelope antigen (B4) of the *E. coli* serotype 0111:B4, but did not have the thermostable somatic antigen (0111) of the strain.

In the pathogenicity study, no chick was infected when the cultures of *A. aerogenes* which possessed B antigen of *E. coli* serotype 0111:B4 and the known strains of pathogenic *E. coli* were given orally, although the organisms fed were recovered from the internal organs of the experimental animals. When the afore-mentioned organisms were given intravenously in 2-week-old chicks, death and symptoms of sickness were observed, as shown in Tables 2, 3, and 4. The chicks inoculated with *E. coli* serotype

TABLE 3

ORAL ADMINISTRATION OF *E. coli* KNOWN SEROTYPES TO 1-DAY-OLD CHICKS

E. coli INJECTED	TESTS								
	After 1 Day			After 5 Days			After 2 Weeks		
	Recovery	Agglutination Test*		Recovery	Agglutination Test		Recovery	Agglutination Test	
		O	B		O	B		O	B
026:56	+	+	+	+	+	−	+	−	−
055:B5	+	+	+	+	+	−	+	−	−
0111:B4	+	+	+	+	+	+	+	−	+
0127:B8	+	+	+	+	−	−	+	−	−
D13†	+	−‡	−‡	+	−‡	−‡	+	−‡	−‡

* Agglutination test: tested with the specific antiserum for each known serotype only. Intestines were considered to be the best source for recovering the organisms administered.

† Control, non-antigenic *E. coli*.

‡ Negative agglutination with all four antisera.

TABLE 4

INTRAVENOUS INJECTION OF ANTIGENIC *A. aerogenes* AND *E. coli* INTO 2-WEEK-OLD CHICKS

ORGANISMS	TESTS							
	After 1 Day			After 5 Days			Effect	
	Recovery of Organisms	Agglutination Test*		Recovery of Organisms	Agglutination Test		Killed	Sick
		O	B		O	B		
A5	+	−	+	−	−	−	1	2
B6	+	−	+	−	−	−	0	3
B11	+	−	+	−	−	−	0	0
D18	+	−	+	−	−	−	0	0
H9	+	−	+	−	−	−	0	1
J15	+	−	+	−	−	−	1	2
E10†	+	−‡	−‡	−	−‡	−‡	0	0
026:B6	+	+	+	−	−	−	3	2
055:B5	+	+	+	−	−	−	1	1
0111:B4	+	+	+	−	−	−	0	3
0127:B8	+	+	+	−	−	−	0	0
D13§	+	−‡	−‡	−	−‡	−‡	0	0

* Agglutination test: *A. aerogenes* were tested with 0111:B4 antiserum; *E. coli* tested with antiserum of its own serotype.

† Control culture of *A. aerogenes*.

‡ Negative agglutination with all four antisera.

§ Control culture of *E. coli*.

0127:B8 and non-antigenic *E. coli* and non-antigenic *A. aerogenes* isolated from human mouths were not affected.

Peculiar colonies were produced on the differential media when the known *E. coli* serotypes were grown together with the isolated *A. aerogenes* for the study of the antigen adaptation. These colonies were not typical of either *E. coli* or *A. aerogenes*. However, the majority of these colonies seemed to resemble *A. aerogenes*. Only a few of such colonies were found to resemble *E. coli*. The biochemical reactions of these intermediate colonies were not typical of *E. coli* or of *A. aerogenes*. Weaker sugar fermentation or sugar fermentation without gas production was observed in several cultures obtained from such intermediate colonies. The differentiation between *E. coli* and *A. aerogenes* for these intermediate forms was rather difficult, and the results were not clear-cut. Atypical reactions on methyl red and Vages-Proskauer tests were especially noticeable. The antigen study showed that some of these cultures were in possession of the thermolabile B antigens, the thermostable O antigens, or both antigens of the known *E. coli* serotypes. *Aerobacter aerogenes*, which were found to possess the thermolabile B antigen of *E. coli* 0111:B4 serotype from the previous experiment, were found to retain the antigenicity when grown together with the four known *E. coli* serotypes. Table 5 shows the results from this study.

The three cultures of non-antigenic *E. coli* isolated from human mouths were not used for this experiment, since it was impossible to distinguish the colonies of these *E. coli* from the colonies of the four known serotypes.

DISCUSSION

Carriers of coliform bacteria were observed during this study. These coliform bacteria were found to be present in certain human mouths among a variety of other oganisms. A rather low incidence of recoveries of this group of organisms from human mouths may be due to the fact that coliform bacteria are primarily the inhabitants of intestinal tracts.

Although during this study none of the isolated *E. coli* was found to belong to any one of the four known serotypes, the human mouth should still be considered as a possible source of infection of infantile diarrhea disease caused by pathogenic *E. coli* serotypes. These serotypes may be found if larger populations are examined.

Because of the limited variety of antisera used, the coliform organisms other than the four known serotypes, which could possibly be present among the isolates, were not detected.

The thermolabile antigen of *E. coli* 0111:B4 serotype seems to be more widely found. This antigen is also occasionally found to be associated with the coliform organisms isolated from various sources such as animals, feeds, water, etc.

In comparison with ordinary *A. aerogenes*, the *A. aerogenes* which were found to possess the thermolabile B antigen of *E. coli* serotype 0111:B4 exhibited some distinguishable differences, such as weaker sugar-fermenting ability and the production of smaller, less swollen, lighter colonies on the differential media used. Some similar characteristics were also noticed on the four known *E. coli* serotypes when they were compared with ordinary *E. coli;* yet none of these characteristics was sufficient in differentiating the non-antigen-possessing cultures from antigen-possessing cultures.

All the four known *E. coli* serotypes, as well as the *A. aerogenes* possessing the B

TABLE 5

BIOCHEMICAL AND ANTIGENIC TEST OF ORGANISMS OBTAINED FROM MIXED GROWTH OF KNOWN *E. coli* AND *A. aerogenes* CULTURES OBTAINED FROM HUMAN MOUTHS

CULTURE NO. OF *A. aerogenes*	TESTS																												
	026:B6							055:5							0111:B4							0127:B8						
	Lc	Gl	Ci	MR	VP	Agglu.		Lc	Gl	Ci	MR	VP	Agglu.		Lc	Gl	Ci	MR	VP	Agglu.		Lc	Gl	Ci	MR	VP	Agglu.	
						O	B						O	B						O	B						O	B
A3	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	−	−	+	−	−
A5*	Ag	Ag	+	+	+	+	−	AG	AG	+	−	+	−	−	ag	aG	+	−	+	−	+	Ag	AG	−	+	+	+	−
B5	Ag	AG	+	+	+	−	−	AG	AG	+	−	+	+	−	AG	AG	+	−	+	−	+	AG	AG	+	−	+	−	−
B6*	Ag	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	+	AG	AG	+	−	+	−	−
B11*	Ag	Ag	+	+	+	+	−	Ag	AG	+	+	+	−	−	AG	AG	+	−	+	−	+	Ag	Ag	+	−	+	−	−
B23	AG	AG	+	−	+	−	−	Ag	AG	+	+	+	−	−	Ag	AG	+	−	+	−	+	AG	AG	+	−	+	−	−
B26	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−
C18	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	+	+	−	−	AG	AG	+	−	+	−	−
D18*	AG	AG	+	+	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	+	AG	AG	−	−	+	−	−
E4	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	−	−	+	−	−	AG	AG	+	−	+	−	−
E10	AG	AG	+	−	+	−	−	AG	AG	+	+	+	+	+	AG	AG	+	−	+	−	−	AG	AG	+	+	+	+	−
E9	AG	AG	+	−	+	−	+	AG	AG	+	−	+	−	−	AG	AG	+	+	+	+	−	AG	AG	−	−	+	−	−
H7	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	−	+	+	−	−	AG	AG	+	−	+	−	−
H9*	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	−	−	+	−	+	AG	AG	+	−	+	−	−
I12	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−
I14	AG	AG	+	+	+	−	−	ag	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	−	+	+	+	+
I16	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−
J5	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−	AG	AG	+	−	+	−	−
J15*	Ag	AG	+	−	+	−	−	AG	AG	+	+	+	+	+	AG	AG	+	−	+	−	+	Ag	AG	+	−	+	−	−

* *A. aerogenes* which possessed B antigen of *E. coli* 0111:B4 serotype. Lc: lactose; Gl: glucose; Ci: citrate; MR: methyl red test; VP: Voges-Proskauer test; Agglu.: agglutination test with the antiserum for the strain of *E. coli*. A: acid; a: little acid; G: gas; g: little gas.

antigen of *E. coli* serotype 0111:B4, showed certain degrees of increased virulence when passed in vivo. The *E. coli* serotype 026:B6 especially, when recovered from chicks following intravenous injection, was found to have a tendency to become hemolytic. Whether this property is predominant in this organism or whether certain animal passages contribute it to the organism is not known.

In many respects, B antigen is similar to Vi antigen of *Salmonella* species. When B antigen is heated at 100° C. for 1 hour, its agglutinability is inactivated, but its antibody-binding power is not destroyed.[3] The boiling or heating is likely to remove the envelope antigen from the bacteria rather than destroy it. In the case of Vi antigen of *Salmonella,* it was shown that heating as well as alcohol removes the antigen from the bacteria by bringing it into solution.[17] Some antigenic interrelationship of certain *Escherichia* to *Shigella* was also recognized by other workers.[18, 19]

Some strains of coliform organisms lose B antigens more readily than others when transferred on artificial media. When a known pathogenic serotype shows the B inagglutinability upon passage on artificial media, it must not be concluded that the organism is no longer pathogenic, even if it does not possess the O antigen which is known to be associated with infantile diarrhea disease. The B inagglutinability in some cases may mean that B antigen is still present but not in sufficient quantity to show agglutination. Consequently, a B-inagglutinable strain may still be pathogenic when the organism gains its way in vivo.

The B antigens of *E. coli* serotypes may also be present in some bacteria other than *E. coli.* These bacteria could have been responsible for the cases of infantile diarrhea disease in which no typical *E. coli* serotypes were found. The acquisition of both envelope (B) and somatic (O) antigens by non-antigen-possessing organisms may be of some significance. When the non-antigen-possessing organisms acquired the antigens of the known *E. coli* serotypes, they became virulent. Although the intermediate forms produced by the mixed growth of the known *E. coli* serotypes and *A. aerogenes* showed some characteristics of both parent types, further genetic studies are necessary for determining whether they were true recombinants or not. Such intermediate forms may also be responsible for the cases of infantile enteritis from which no *E. coli* was recovered.

It should be realized that difficulties are encountered in studying the pathogenicity of the enteropathogenic coliform organisms. Although young chicks were used in this study, several coliform-like organisms with some *E. coli* antigens were isolated from the chick feed. The isolation of *E. coli* serotypes, especially 0111:B4 from fowls, has been pointed out by Edwards,[20] and the isolation of 0111a:B4 serotype from monkeys has been studied by Ewing, Galton, and Tanner.[21] Pathogenicity studies should not depend entirely on the effect on chicks, because chicks may develop resistance as they grow.

SUMMARY

Twenty-two cultures (8.6 per cent) of coliform bacteria were isolated from a group of 254 adult human mouths studied. Among these, 3 cultures (1.2 per cent) were *E. coli,* and 19 cultures (7.4 per cent) were *A. aerogenes.*

Four known *E. coli* serotypes were used as controls for studying the antigens and the pathogenicities of the isolates. No antigens were found in the *E. coli* isolated from

the mouth. However, six cultures of the isolated *A. aerogenes* were found to possess B antigen of *E. coli* serotypes 0111:B4. The pathogenicities of these *A. aerogenes* cultures compared favorably with *E. coli* serotype 0111:B4.

Some offspring resulted from the mixed culture of the known *E. coli* serotypes, and the isolated *A. aerogenes* presented atypical morphologic, biochemical, and serologic characteristics.

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Localization of Calcium Placed over Amputated Pulps in Dogs' Teeth

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Calcium hydroxide has been used successfully as a dressing over exposed dental pulps and after pulp amputations to promote the formation of a dentin bridge in the teeth of humans, dogs, and rats.[1] A good deal of clinical and experimental evidence has accumulated to show that this material causes the formation of a dentin roof or bridge over the denuded pulp more quickly than do most other substances.[2, 3] In spite of extensive studies, it is not yet clear whether the rapidity of the bridging is due to its acid-neutralizing action, to the calcium ion, or even to its coagulating powers. Zander[4, 5] believes that calcium diffuses from the calcium hydroxide dressing into the pulp and enters into the formation of the new dentin roof. Others suggest that the calcium necessary for the calcification of the new dentin is derived via the vascular supply from the pulp, since good dentin bridging can be observed under dressings which do not contain calcium.[2]

The purpose of this study was to determine whether the calcium from the calcium hydroxide dressing entered into the formation of the new dentin roof. Radioactive calcium was used to trace whether a diffusion of calcium ions occurs from the calcium hydroxide placed over amputated pulps into the dentin bridge or pulp of dogs' teeth.

MATERIALS AND METHODS

Forty-two teeth in two dogs were prepared for this experiment. Both dogs were well-nourished males, approximately $2\frac{1}{2}$ years old and weighing about 7 kg. Their condition remained good throughout the duration of the experiment.

The animals were anesthetized with sodium pentobarbital injected intraperitoneally at 30 mg/kg. The teeth and gingivae were swabbed with a 5 per cent solution of iodine in alcohol. Cavities were prepared on the buccal surfaces of the canine and molar teeth approximately 1.0 mm. above the free gingival margin, using tungsten carbide burs revolving at 3,000 rpm. Drilling was stopped at the first appearance of blood following penetration of the pulp. As a rule, bleeding stopped of its own accord. When it continued for any length of time, it was controlled by application of a saturated solution of calcium hydroxide in distilled water. The exposed pulp was then covered with a paste of calcium hydroxide (pH 10.5–12.5) containing 120 microcuries of radioactive calcium ($Ca^{45}Cl_2$) per gram of calcium hydroxide powder. Each dressing was then covered with a zinc oxide–eugenol cement, care being taken to avoid pressure on the pulp.

Three teeth were treated each week in each dog for 6 weeks. The dogs were sacrificed by an intravenous injection of air into the femoral vein, 2 weeks after the last treatment, so that a total of 6 teeth was available for histologic study after intervals of 2, 3, 4, 5, 6, 7, and 8 weeks after pulp capping. The jaws were separated from the skull and

fixed in 10 per cent neutral formalin for 1 week. Roentgenograms were taken of all teeth. Two teeth (7 and 6 weeks postoperative) were prepared by routine histologic methods (decalcified in 5 per cent nitric acid and imbedded in celloidin-paraffin), cut into serial sections, and stained with hemotoxylin-eosin and azan. All other teeth were imbedded* over a period of 2 weeks and sliced into slabs approximately 0.5 mm. thick. The slabs were then ground and polished into sections approximately 50 μ thick. Autoradiographs were obtained from each section by pressing it tightly against the emulsion side of intra-oral dental films† for 36–72 hours.

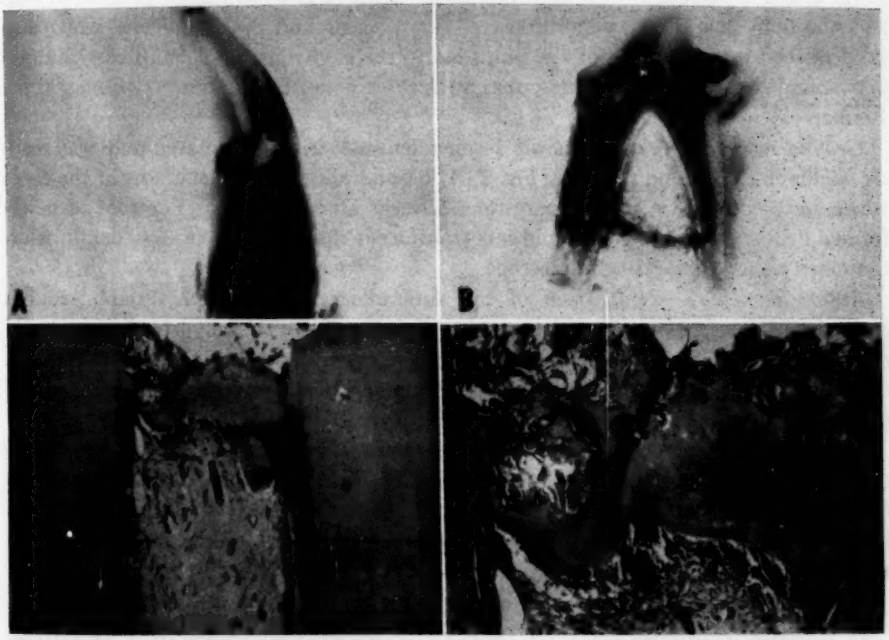

FIG. 1.—*A:* Roentgenogram—canine. Two cavities were prepared. The temporary filling is radiopaque. The cavity containing the $Ca(OH)_2$ is radiolucent. Opaque dentinal bridges are visible in the coronel as well as the radicular side of the pulp. *B:* Same as *A*—molar. The occlusal cavity shows, under the $Ca(OH)_2$-containing radiolucent area, a distinct dentinal roof. *C:* Decalcified section—canine. H.E. Occ. × 6; obj. × 2.5. This section shows a new dentin roof formed over the pulp 6 weeks after amputation and covering with Ca hydroxide paste containing Ca^{45}. The pulp tissues on the radicular side show an engorgement of the blood vessels and some infiltrating cells. Note secondary dentine formation all along the root walls. *D:* Same as *C*. Occ. × 6; obj. × 12. The new secondary dentin roof is formed by regular canalicular dentin. The thin dentinal wall on the left side is covered by very irregular dentin, interspersed with necrotic debris and often formed around dentin splitters.

RESULTS

Decalcified sections.—The two teeth which were decalcified and prepared in stained sections showed the same picture as already described by Zander,[4, 5] Nyborg,[3] and others.[2] Under the space occupied by the calcium hydroxide dressing there was a large area filled with necrotic material and debris (Fig. 1, *C, D*). Under this area was a nar-

* Bio-Plastic, manufactured by Ward's Natural Science Establishment Inc., Rochester, New York, was used.

† Kodak ultra-speed film was used.

row zone of basophilic fibrous tissue containing large cells and occasional dentin chips. This zone has been described previously as the zone of dystrophic calcification or temporary dentin bridge[2, 3] (Fig. 1, *C, D*). Under this area was a well-formed thick roof or bridge of regular dentin (Fig. 1, *C, D*). The dentin roof over the pulp was acidophilic and tubular. New dentin of a similar character was also deposited along the walls of the old dentin lateral to the site of bridging.

The roof of new dentin was lined by a layer of newly differentiated odontoblasts, under which there were many engorged blood vessels and frequent small hemorrhages (Fig. 1, *C*). Underneath, cell infiltration was common and prominent (Fig. 1, *D*). In some sections new dentin deposition could be seen around dentin spicules which had been pushed into the body of the pulp during the cavity preparation. These findings corresponded closely to those described in greater detail by Zander,[4, 5] Nyborg,[3] and Berman.[1, 2]

Ground sections.—A roof of newly formed dentin over the amputated pulp was readily visible in the ground sections (Fig. 2, *A, B*) and in the roentgenograms of the teeth which were allowed to survive more than 3 weeks after operation. The roof of newly formed dentin was more yellow and less translucent than the surrounding dentin when examined under a dissecting microscope.

Autoradiographs.—Comparison of the autoradiographs with the ground sections showed that the area of radioactivity was confined to the calcium hydroxide dressing in every specimen (Fig. 2, *C, D*). There was no evidence of radioactivity within the area of new dentin formation (Fig. 2, *E, F*). Some sections showed a slight diffusion of Ca^{45} along the dentino-enamel junction (Fig. 2, *C, D*).

Drawings of the blackened area in the radioautograph were superimposed upon photographs of the ground sections. In each instance this showed that the Ca^{45} was confined to the calcium hydroxide dressing (Fig. 2, *E, F*). Radioactivity did not appear in the area of new dentin covering the pulp, even in autoradiographs exposed for as long as 3 weeks.

DISCUSSION

Many clinical and experimental studies indicate that the application of calcium hydroxide over the exposed or amputated pulp promotes the rapid formation of a calcified bridge or roof of new dentin.[1] The specificity of this action has been questioned, since the formation of new dentin can occur under dressings other than calcium hydroxide.[2] However, there is little doubt that dentin formation occurs more rapidly under calcium hydroxide than under other dressings.[3] Some authors suggest that this may be due to the high pH, which may favor the precipitation of calcium salts.[1] Others indicate that this may be due to the coagulation of the pulp in contact with the calcium hydroxide.[2] The present study indicates that the calcium ions from the calcium hydroxide do not enter into the formation of the new dentin roof. The calcium ions necessary for the calcification of new dentin are therefore probably derived from the pulp itself.

SUMMARY

Forty-two teeth in two dogs were subjected to pulp amputations. The exposed pulps were covered with a paste of calcium hydroxide containing radioactive calcium (Ca^{45}). New dentin roofs were formed over the exposed pulps in every tooth examined 3 or

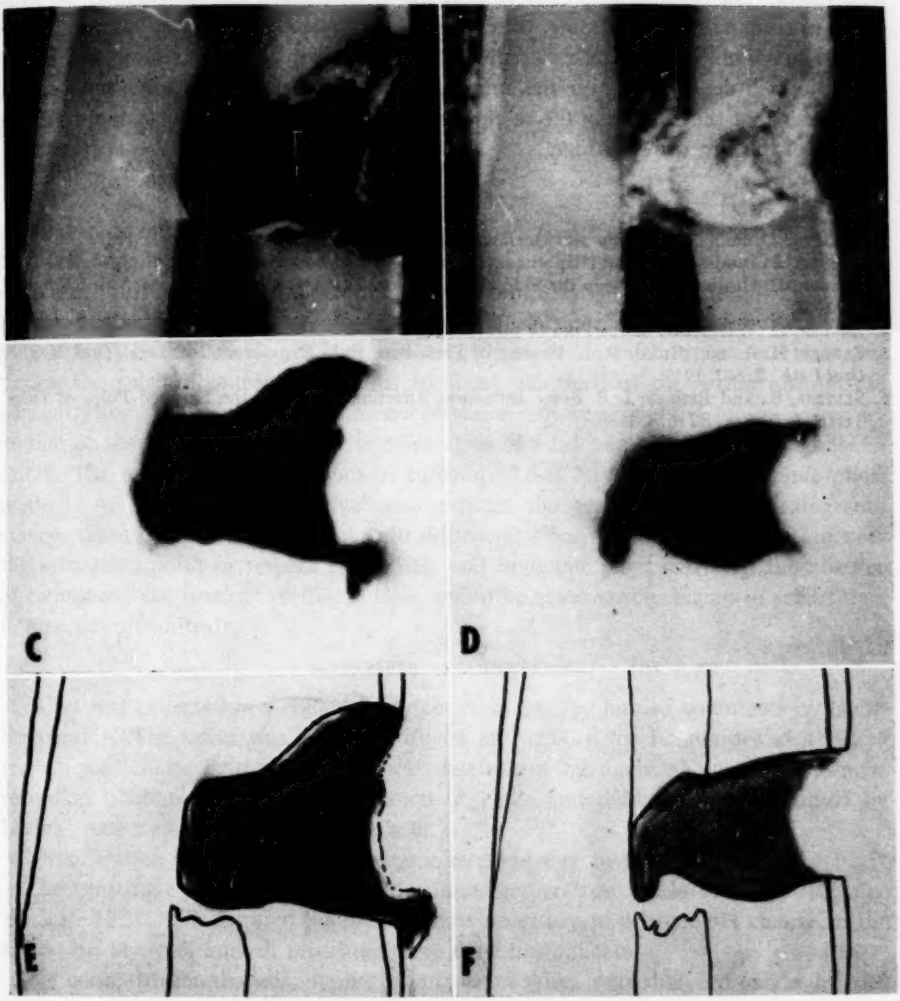

FIG. 2.—Linear magnification × 10. *A* and *B:* ground section—canine. Distinct dentinal bridge covering pulp. In *A* the radioactive calcium hydroxide fell out during manipulation. The zinc oxide–eugenol cover remained. In *B,* both materials are intact. *C* and *D:* Autoradiographs of *A* and *B*. The contours are distinct and do not include the dentin bridge. There is some diffusion of radioactive material into the coronel dentin and the dentino-enamel function. *E* and *F:* The picture shows, on the same scale, a linear drawing of the ground section, superimposed on the radioautograph. The dentinal bridge is free from radioactivity.

more weeks after operation, in a manner previously described by Zander,[4] Nyborg,[3] and others.[2] Radioautographs showed that the radiocalcium was confined to the area of the calcium hydroxide dressing, with some slight diffusion along the adjacent dentinoenamel junction. The presence of radiocalcium could not be demonstrated within the newly formed dentin roof over the amputated pulp. This study does not support the hypothesis that the calcium in the calcium hydroxide enters into the formation or calcification of the new dentin bridge.

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Calcium Binding by Normal Human Saliva

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A number of findings indicate the possibility that a part of the calcium in saliva is bound or complexed in non-ionic form. For example, the salivary glands of rats contain more calcium than do many other soft tissues, and a large portion of this calcium is lost during secretion of saliva. As secretion is initiated in rats by administration of pilocarpine, the saliva calcium is almost as high as serum calcium; as secretion continues, the calcium concentration falls to about one-third of the serum ultrafiltrate concentration.[1] This fall in calcium concentration can be accounted for by active secretion or absorption or by falling concentration of a calcium-complexing substance in saliva. The reported concentrations of calcium (1.3–1.7 mM/l) and inorganic phosphate (1.93–8.71 mM/l) in saliva[2] also indicate the possibility of complexing substances when compared with serum ultrafiltrates,[3] since serum ultrafiltrates are near the saturation point in respect to calcium and inorganic phosphate.[4] In the absence of complexes, the product of $C_{Ca} \times C_{PO_4}$ would be considerably higher in saliva than in a serum ultrafiltrate.

MATERIALS AND METHODS

Saliva was collected in a chilled container from healthy human volunteers by chewing paraffin. The saliva was then centrifuged at 10,000 g for 10 minutes at 4° C. to remove particulate matter. Calcium was determined by flame photometry,[5] using a recording photometer,[6] while the method of Fiske and SubbaRow[7] as modified by Loring[8] was used for inorganic phosphate.

Ultrafiltration in a 5 per cent CO_2 atmosphere was done by the method of Toribara.[9]*

The centrifuge was at 4° C., and the final temperature inside the filtration tube was 13°–15° C. The lowered temperature was necessary to prevent pH change in the saliva. All analyses and all filtrations were done in duplicate.

For concentration dialysis, duplicate samples of saliva were dialyzed overnight with stirring at 4° C. against 20 per cent polyvinyl pyrrolidone (PVP) containing 0.43–1.89 mM $CaCl_2$/l. Calcium in solution with PVP was found to be completely filterable, and the Ca^{++} concentration was presumed to be the same on both sides of the dialysis membrane.

The total concentration of calcium-complexing material in saliva as compared with sodium citrate was determined by the method of Schubert.[10] Approximately 10 mg.

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* Visking 23/32 Nojax casing was used.

of cation-exchange resin* (100–200 mesh, 2.5 mM/gm exchange capacity, sodium form), weighed to the nearest 0.01 mg., was used. The tracer was Ca^{45}-P3 with specific activity of approximately 1,500 mc/gm.† Equilibration between resin and solution was brought about by shaking in 16 ml. teflon-capped vials for 8 hours at 4° C. Each vial contained 2.0 ml. of 0.05 M sodium barbital buffer at pH 7.4. The solution used for comparison was 0.02 M citric acid (reagent grade) adjusted to pH 7.4 with NaOH. After preliminary experiments, the following procedure was used. Centrifuged saliva was first passed through a 16–50-mesh sodium-form cation resin exchange column (2 × 11 cm.)‡ to remove calcium. Before use, the column was washed with ion-free water from a research model de-ionizer§ until the effluent was below 10 μEq Na/l. At least 30 ml. of saliva were allowed to pass through the column before samples for analysis were collected, in order to prevent dilution of saliva samples by wash water on the column. The calcium concentration of the effluent saliva was below 50 μEq/l, and the inorganic phosphate concentration was not changed. The exchange capacity of 10 mg. resin‖ was approximately 50 μEq cation, and the presence of less than 0.5 μEq calcum in a saliva sample was considered to have no appreciable effect on the equilibrium. The sodium concentration of the calcium-low saliva was determined by direct-flame photometry, and all samples and citrate standards were adjusted to a sodium concentration of 0.06 molar by the addition of NaCl solution. Final volume for all vials was 10 ml. After equilibration, the radioactivity of the solution was determined by thin-window GM counter after plating 0.1 ml. on copper planchets. The amount of radioactivity in the resin was calculated by difference.

RESULTS

1. *Ultrafiltration and dialysis.*—The results of saliva ultrafiltrations are shown in Table 1. The calcium in saliva is only partly filterable. The range of the non-ultrafilterable calcium was from 4 to 20 per cent, the mean was 12.8 per cent, and the standard error was ±1.1. Non-ultrafilterable inorganic phosphate ranged from 2 to 13 per cent, with a mean of 5.7 per cent and a standard error of ±0.5. The non-ultrafilterability of a portion of the saliva calcium indicated the possibility either of the presence of a non-filterable calcium-binding molecule or of the presence of a precipitate or colloidal aggregate of calcium. To differentiate these possibilities, the same saliva samples were concentrated against a PVP solution containing calcium approximately equivalent to the saliva filtrate but low in inorganic phosphate, sulfate, and other anions which might form calcium precipitates or aggregates. Thus, if the saliva contained a non-ultrafilterable calcium-binding molecule, the concentration of calcium inside the dialysis membrane should be considerably higher after removal of water than the dialysate, since the calcium-binding molecule would be concentrated. On the other hand, if non-ultrafilterability of calcium resulted from the presence of a precipitate with a filterable anion, then equilibrium between anion inside and anion outside should occur. This process would reduce the amount of anion available to precipitate

* Dowex 50.

† Union Carbide Nuclear Corp., Oak Ridge, Tenn.

‡ Amberlite IRA-120.

§ Illco-way.

‖ Dowex 50.

calcium, and the concentration of calcium inside should be similar to the calcium concentration outside the dialysis membrane. The data of Table 2 indicate that when the non-dialyzable portion of saliva was concentrated from 2.5 to 5.5 times, the percentage of calcium bound averaged 18 per cent, as compared with 12.8 per cent which was non-ultrafilterable. If the calcium bound in concentrated saliva (Table 2) is calculated on the basis that the non-ultrafilterable calcium in saliva (Table 1) results entirely

TABLE 1

ULTRAFILTRATION OF SALIVA

Subject*	Calcium Concentration			Inorganic Phosphate		
	Saliva (mM/l)	Ultrafiltrate (mM/l)	Nonfilterable (Per Cent)	Saliva (mM/l)	Ultrafiltrate (mM/l)	Nonfilterable (Per Cent)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
A	1.50	1.24 1.26	17 16	3.67	3.48 3.53	5 5
A	1.35	1.22 1.22	10 10	3.99	3.74 3.83	6 4
B	1.32	1.18 1.16	11 12	3.63	3.45 3.40	5 6
B	1.37	1.17 1.17	15 15	3.54	3.33 3.30	6 7
C	1.43	1.22 1.18	15 17	4.17	4.00 4.09	4 2
D	1.25	1.00 1.00	20 20	3.53	3.24 3.08	8 13
E	1.42	1.36 1.35	4 4	4.58	4.51 4.49	2 2
F	1.41	1.26 1.27	11 10	3.85	3.54 3.54	8 8
G	1.65	1.45 1.45	12 12	3.85	3.75 3.54	3 8
Mean (±S.E.)	1.41±.04	1.23±.04	12.8±1.1	3.87±.12	3.80±.10	5.7±.5

* Subjects are designated the same in all tables.

from a non-ultrafilterable binding substance which obeys the thermodynamic dissociation equation,

$$K = (Ca^{++}) \frac{(\text{binding substance}^{=})}{(\text{Ca-binding substance})}, \quad [4]$$

then a large difference between Ca_{bound} (measured) and Ca_{bound} (calculated) appears*

* The method of calculation is as follows. Since Ca_{bound}/Ca_{free} remains constant if the amount of binding substance is not changed, then $Ca_{bound^1}/Ca_{free^1} \times Ca_{free^2} = Ca_{bound^2}$. This equation will give the Ca_{bound^2} if the only change is in the concentration of Ca. If the concentration of binding substance is changed, then the calculated amount of Ca_{bound} would be $Ca_{bound^2} \times$ concentration ratio. For these calculations Ca_{bound^1} is the difference between cols. 2 and 3 (Table 1), Ca_{free^1} is col. 3 (Table 1), Ca_{free^2} is col. 4 (Table 2), and the concentration ratio is col. 7 (Table 2).

(Table 2), except in one subject (E, Table 2). Thus only a small portion of the non-ultrafilterability of calcium in saliva (Table 1) is apparently explainable on the basis of the presence of a non-ultrafilterable binding substance.

2. *Complexing capacity.*—The total calcium-complexing capacity in various saliva samples as compared with citrate is shown in Table 3. The reciprocal of the distribution coefficient, $1/K_d$, was calculated for the citrate standards and for the saliva by the equation

$$\frac{1}{K_d} = \frac{\%\text{ radioactivity in solution}}{\%\text{ radioactivity in resin}} \times \frac{\text{mass resin}}{\text{volume solution}}.$$

When $1/K_d$ values for citrate were graphed, a linear plot resulted. The citrate-equivalent complexing capacity could then be determined from this graph, using the $1/K_d$ values for the saliva samples (Table 3). The reproducibility of the method is satisfactory for citrate, but for saliva samples the variation between duplicate samples was 5–10 per cent, and for determinations on different days the maximum variation between samples from the same subject was as much as 40 per cent. Ten determinations were also made on these same subjects by a different technique of saliva preparation. The saliva, after removal of calcium, was concentrated to approximately one-fourth the original volume in a rotary evaporator before analysis of complexing capacity. By this technique, the values for complexing capacity were in most instances less (Table 4) than those obtained when unconcentrated saliva was used (Table 3). The reason for the differences between the values found after concentration and those found when unconcentrated salivas were used is unknown. The final pH during equilibration with

TABLE 2

CONCENTRATION DIALYSIS OF SALIVA AGAINST POLYVINYL PYRROLIDONE

Subject	Calcium Concentration					Conc. Ratio
	Concentrated Saliva (mM/l)	Dialysate (mM/l)	Bound (Measured)* (mM/l)	Per Cent	Bound (Calculated)† (mM/l)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
A	2.25 2.30	1.70	0.55 0.60	24 26	1.15 1.85	5.5 5.5
B	0.45 0.44	0.43	0.02 0.01	4 2	0.22 0.30	4.0 4.3
C	2.23 2.08	1.89	0.34 0.19	15 9	2.8 2.3	3.8 3.2
E	1.80 1.64	1.27	0.53 0.37	29 23	0.20 0.18	2.8 2.5
F	1.61 1.55	1.17	0.44 0.38	27 25	1.4 1.3	4.9 4.6
Mean	.....	.....	.....	18	.....	.....

* Column 2 less column 3.
† See text.

TABLE 3

TOTAL CALCIUM-COMPLEXING CAPACITY OF SALIVA
(Ionic Strength, 0.06; Temp., 4° C.; pH, 7.4)

Experiment No.	Citrate			Saliva		
	μM	$1/K_d \times 10^{-3}$	Subject	Ml.	$1/K_d \times 10^{-3}$	Ca-complexing Capacity (mM/l)*
I	0	0.059	B	8	0.70	1.90
	0	0.058		8	0.64	1.73
	10	0.47	C	8	0.70	1.90
	10	0.48		8	0.63	1.71
	20	0.90	F	8	0.62	1.68
	20	0.91		8	0.66	1.80
			G	8	0.72	1.98
				8	0.76	2.09
II	0	0.11	A	8	0.48	1.00
	0	0.10		8	0.47	0.98
	5	0.34	B	8	0.66	1.48
	5	0.32		8	0.76	1.60
	20	1.08	D	8	0.95	2.20
	20	1.06		8	1.05	2.45
			E	8	0.44	0.90
				8	0.38	0.75
III	0	0.14	A	8	0.57	0.95
	0	0.16		8	0.65	0.92
	5	0.41	D	5	0.64	1.66
	5	0.44		5	0.59	1.52
	20	1.41	E	8	0.36	0.56
	20	1.40		8	0.34	0.50
			$Na_xH_xPO_4$† (mM/l)			
			1.25	8	0.15	>0.05
			1.25	8	0.18	>0.05
			6.25	8	0.22	0.15
			6.25	8	0.22	0.15

* Complexing capacity expressed as citrate equivalent.
† Buffer pH 7.4 phosphate concentration checked by analysis.

TABLE 4

CALCIUM-COMPLEXING CAPACITY OF CONCENTRATED SALIVA

Subject	Saliva-complexing Capacity*
A	0.72, 0.55, 0.60, 0.85
B	0.65
C	0.62
D	2.20
E	0.44
F	1.02, 1.12

* Expressed as citrate equivalent of original saliva.

resin was not changed by the concentration procedure. Loss of all CO_2 from saliva during concentration would not be sufficient to account for the fall in complexing capacity.[11]

The range of total calcium-complexing capacity in different salivas is considerably larger than the range in calcium or inorganic phosphate content (Table 1), and there was no apparent correlation between calcium and inorganic phosphate content and total calcium-complexing capacity. In Experiment 3, Table 3, the calcium-complexing capacity for inorganic orthophosphate was determined, and the capacity for a 6.25 mM/l solution was found to be equivalent to 0.15 mM/l citrate. This result agrees with that of Gosselin and Coghlan.[12]

TABLE 5

SOLUBILITY OF CALCIUM IN SALIVA AND IN INORGANIC PHOSPHATE MEDIA AFTER EQUILIBRATION FOR 16 HOURS AT 4° C. WITH COLLOIDAL $Ca_x(PO_4)$ AT pH 7.4*

SOLUTION	CONC. BEFORE EQUILIBRATION		CONC. AFTER EQUILIBRATION	
	Calcium Chloride (mM/l)	Sodium Phosphate (mM/l)	Calcium (mM/l)	Inorganic Phosphate (mM/l)
0.05 M NaCl.....	1.5	3.5	0.42	2.86
	1.5	3.5	0.42	2.93
	1.5	4.0	0.34	3.05
	1.5	4.0	0.33	3.15
	1.5	4.5	0.30	3.65
	1.5	4.5	0.25	3.54
Saliva.....	1.47	3.89	0.93	3.18
	1.47	3.89	0.92	3.30

* Precipitated from 1.5 mM Ca/l, 3.5 mM phosphate/l.

3. Evidence of calcium complexing by saliva was also found by equilibrating normal saliva (subject A) and solutions of calcium chloride and sodium phosphate at pH 7.4 with a colloidal precipitate of $Ca_x(PO_4)_x$. This precipitate was obtained from a solution originally containing 1.5 mM/l calcium and 4.0 mM/l inorganic phosphate buffered at pH 7.4. The final concentrations of calcium and inorganic phosphate for a typical experiment are given in Table 5. Almost three times as much calcium was retained in solution in saliva as in the inorganic media having the same initial concentrations of calcium and phosphate.

DISCUSSION

The evidence indicates that a considerable portion of the calcium in saliva is in associated form, although the nature and magnitude of the association cannot be stated with the present information. At least a part of the binding appears to be on the basis of a non-ultrafilterable molecule, since the average non-dialyzable calcium was 18 per cent after saliva was concentrated 2.5–5.5 times against PVP. The known protein content of saliva (4 gm/l) could account for approximately one-third of the non-

dialyzable calcium if the protein of saliva has the same calcium affinity as serum protein.

If saliva is ultrafiltered through a collodion membrane, an average of 12.8 per cent is non-ultrafilterable. A large portion of this non-ultrafilterability probably results from aggregation of calcium with phosphate or other small anions into particles too large to pass the membrane, since concentration of saliva against PVP does not result in an increase in bound calcium proportional to the concentration ratio. This procedure allows diffusion of small anions into the dialysate in the direction of the concentration gradient. Thus phosphate, sulfate, or other small, filterable binding anions can escape because the concentration of these anions outside the membrane is much lower than that inside.

When the calcium-complexing capacity of saliva was measured by equilibration against a cation-exchange resin, the complexing capacity as compared with citrate for 7 subjects was found to vary from 0.75 to 2.45 mM/l. These values range from approximately half to almost twice the calcium concentration of saliva. The complexing capacity did not correlate directly with the calcium or phosphate content of the saliva, and, in fact, the subject with the lowest calcium-complexing capacity had the highest inorganic phosphate concentration (subject E, Tables 1 and 3). These data indicate that, under the conditions of the equilibrium experiment, the complexing capacity of saliva appears to be sufficient to bind nearly all the calcium in most subjects, since 1.8 mM/l of citrate will bind approximately 1.2 mM/l of calcium.[4] The use of citrate as a reference in these experiments was necessary because the compound or compounds responsible for the binding are unknown and hence a formation constant could not be calculated in the usual way. The use of a secondary reference was considered to be justified, in order to obtain an estimate of the total quantity of calcium bound, even though an error might be introduced. The various organic substances in saliva could also prevent or delay the equilibration of Ca^{45} with the ion-exchange resin in some other way and give rise to the observed calcium-complexing capacity without actually forming a true complex such as occurs with citrate. Unfortunately, however, the ionic calcium in normal saliva cannot be determined from these data for the following reasons. Cations other than sodium were removed from the test sample, and the complexing capacity was determined in the presence of only trace amounts of calcium. The ionic strength of the final equilibration solution was also adjusted. The temperature of equilibrium was also lowered to prevent enzymatic and bacterial decomposition of the saliva insofar as possible. These procedures changed the equilibrium to an unknown extent. No method appears to be usable for determining directly the Ca^{++} concentration in solutions such as saliva. The frog-heart method of MacLean and Hastings[13] was tested briefly but required adjustment of sodium and potassium concentration. The results indicated that the Ca^{++} concentration was probably less than 0.5 mM/l when the total calcium was 1.5 mM/l.

The substances responsible for the complexing of saliva in calcium are unknown. Non-dialyzable material apparently accounts for 5–10 per cent of the total, and inorganic phosphate and carbonate could account for a similar amount. The remainder is apparently dialyzable and hence is composed of relatively small molecules. This complexing capacity is apparently not limited to the saliva, since, at least in rats, the salivary glands are able to maintain a large calcium concentration gradient against extra-

cellular fluid in vivo.[1] After disruption, the particulate fractions of the submaxillary gland of rats are also able to maintain a high calcium concentration as compared with the media.[14]

SUMMARY

Normal human saliva from most subjects contains substances apparently capable of complexing more than half the calcium present. The inorganic phosphate and protein of saliva are present in insufficient quantities to account for more than a small fraction of the calcium-complexing capacity. Evidence indicates that most of the complexing material is dialyzable.

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Blood Flow and Blood Pressure in the Internal Maxillary Artery

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Blood-flow measurements have been used for physiologic studies of the circulatory response to fluctuating tissue demands which occur intrinsically, follow disturbances of the circulation, or develop after pharmacologic treatment. The development and improvement of blood-flow techniques[1–3] has made it practical to measure small volume flows. Recently the average blood flow, blood pressure, and peripheral resistance, along with some of the factors affecting these parameters, have been determined in the mandibular artery of dogs.[4]

Since measurement of blood flow to the oral region has been largely neglected,[5, 6] the purpose of this investigation was to determine the average blood flow and blood pressure in the internal maxillary artery of dogs. The effect of sympathomimetic and parasympathomimetic drugs on the peripheral vascular bed was studied.

EXPERIMENTAL METHODS

Unilateral blood-flow and blood-pressure measurements were made in the right internal maxillary arteries of 12 mongrel dogs. The animals were anesthetized with 33 mg/kg sodium pentabarbital given intravenously. Five mg/kg of heparin were used to prevent blood coagulation. The internal maxillary artery was exposed by an inframandibular incision, and a polyethylene tube, with bore size comparable to the lumen of the artery, was inserted into the artery in such a way as to form an extravascular loop. This arrangement permitted the blood to flow through the field of an electromagnetic blood flowmeter.[1, 2] Volume blood flow was then recorded on an ink-writing oscillograph. Blood pressure was simultaneously recorded by the use of a strain gauge, a strain analyzer, and an ink-writing oscillograph.

Peripheral resistance was calculated in terms of peripheral resistance units (PRU) by dividing the pressure in millimeters Hg by the blood flow in cubic centimeters per minute.[7] Effective doses of sympathomimetic and parasympathomimetic drugs were injected into the extravascular loop distal to the electromagnetic blood flowmeter. Radiographs were made during injection of thorium dioxide into the distal end of the polyethylene cannula.

RESULTS

Analysis of the radiograph (Fig. 1) indicates the relatively large area supplied by the internal maxillary artery and its branches in the dog. The maxilla, nasal area, and orbital region all receive well-defined branches.

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The mean blood flow in the internal maxillary artery was found to be 19.5 cc/min ± 1.9, and the mean blood pressure was 114 mm. Hg ± 3.8. The mean value in peripheral resistance units was 7.1.

Figure 2 is a typical recording of arterial blood pressure and internal maxillary artery blood-flow changes which occurred in response to 1.0 μg. acetylcholine. Blood pressure decreased from the control value of 150–130 mm. Hg, and the blood flow increased from 11.8 to 16.5 cc/min. Following injection of 0.1 μg. of epinephrine, an active vasoconstriction occurred (Fig. 3). The blood pressure increased from 150 to 175 mm. Hg and the blood flow decreased from 12.2 to 6.8 cc/min.

FIG. 1.—Radiograph of internal maxillary artery following thorium dioxide infusion

Table 1 summarizes the cardiovascular data obtained from twelve experimental animals in response to vasomotor drugs. Ten seconds after the injection of 0.1 μg. epinephrine, blood flow decreased approximately 60 per cent, and blood pressure increased about 15 per cent. Peripheral resistance more than doubled, which indicated an active vasoconstriction. Ten seconds after administration of 1.0 μg. acetylcholine, mean blood flow increased approximately 43 per cent, arterial blood pressure decreased about 8 per cent, and peripheral resistance was decreased by 36 per cent.

DISCUSSION

Radiographic studies, following thorium dioxide infusion, indicate an extensive tissue bed supplied by the internal maxillary artery (Fig. 1). Since volume flow is directly proportional to mean velocity flow times cross-sectional area,[8] an increased cross-sectional area would increase volume flow if the mean velocity remained constant.

Because of the diffuse area supplied by the internal maxillary artery, it was not practicable, under the conditions of this investigation, to determine volume blood flow per unit of tissue weight; however, a relative comparison might be made between the data from the internal maxillary artery and that previously reported for the mandibular artery.[4] Mean mandibular blood flow was reported to be 6.0 ± 0.6 cc/min, which is approximately one-third as much as internal maxillary volume flows. Arterial blood pressure was not significantly different in the two arteries. Peripheral resistance in the internal maxillary artery was found to be only 35 per cent as much as in the mandibular artery. However, the vascularity of the tissues supplied by the internal maxillary artery may well be no greater than that which obtains for the mandibular tissues,

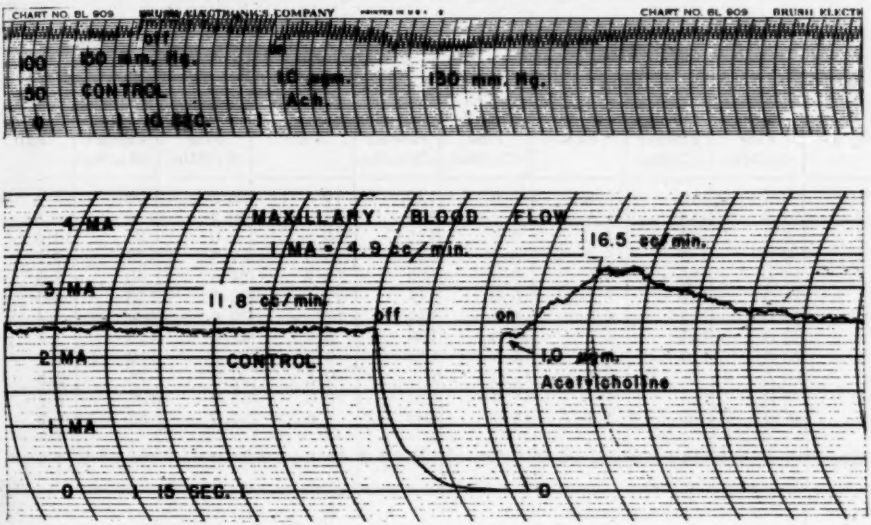

FIG. 2.—Blood pressure in mm. Hg (*upper record*) and blood flow in cc/min (*lower record*) in response to 1.0 μg. acetylcholine injected into the internal maxillary artery.

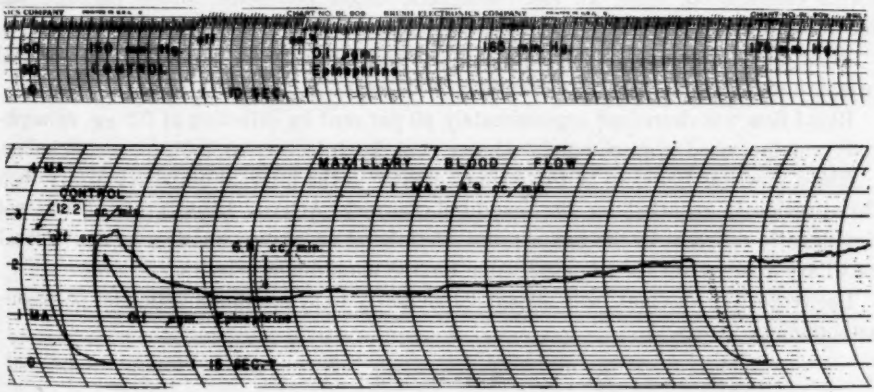

FIG. 3.—Blood pressure (*upper record*) and blood flow (*lower record*) changes in response to 0.1 μg. epinephrine.

since the area supplied by the internal maxillary artery is viewed as being sufficiently large to account for the larger blood flow through the maxillary artery.

Adrenergic and cholinergic drugs produced vasoconstriction and vasodilatation, respectively, in each vascular bed. Epinephrine decreased mandibular flow 60 per cent and decreased maxillary flow 50 per cent. Acetylcholine increased mandibular flow 33 per cent and increased maxillary flow 43 per cent. These data indicate that there exists the same basic type of chemical mediation in these two areas.

TABLE 1

AVERAGE BLOOD-FLOW AND BLOOD-PRESSURE DATA SHOWING CHANGES IN RESPONSE TO EPINEPHRINE AND ACETYLCHOLINE

Dog No.	Control			10 Sec. after 0.1 μg. Epinephrine			10 Sec. after 1.0 μg. Acetylcholine		
	Blood Flow (Cc/Min)	Blood Pressure (Cc/Hg)	PRU	Blood Flow (Cc/Min)	Blood Pressure (Mm/Hg)	PRU	Blood Flow (Cc/Min)	Blood Pressure (Mm/Hg)	PRU
1.....	9.5	110	11.6	7.0	130	19	16.0	90	5.6
2.....	13.5	155	11.5	4.7	170	37	20.3	135	6.6
3.....	6.5	95	14.6	0.5	125	250	8.0	85	10.6
4.....	9.5	145	15.3	6.2	152	25.5	17.5	135	7.7
5.....	13.0	105	8.1	7.0	100	14.3	24.0	90	3.8
6.....	17.0	120	7.1	6.0	135	22.5	28.0	120	4.3
7.....	24.0	115	4.8	8.0	135	17	29.0	100	3.4
8.....	32.0	145	4.5	22.0	150	6.9	45.0	125	2.8
9.....	20.8	120	5.8	14.5	135	9.3	27.2	110	4.0
10.....	18.5	85	4.6	11.0	105	9.5	29.5	75	2.6
11.....	28.0	105	3.7	13.0	130	10.0	38.0	105	2.7
12.....	38.0	85	2.2	18.0	100	5.6	54.0	85	1.6
Mean.	19.5±1.9	114±3.8	7.1±0.9	9.7±1.7	131±6.2	16.8±2.8	28.0±3.7	105±5.9	4.6±0.7

SUMMARY

Blood flow in the internal maxillary artery of anesthetized dogs was measured by direct cannulation, using an electromagnetic blood flowmeter. Blood pressure was recorded by the use of a strain gauge, strain analyzer, and ink-writing oscillograph.

Mean blood flow in the internal maxillary artery was 19.5 cc/min, and mean blood pressure was 114 mm. Hg. The calculated PRU was 7.1.

Blood flow was decreased approximately 60 per cent by injection of 0.1 μg. epinephrine and increased approximately 43 per cent by injection of 1.0 μg. acetylcholine.

Blood flow was found to be three times greater in the internal maxillary artery than that previously reported for the mandibular artery. Arterial blood pressure was not significantly different in the two arteries. Peripheral resistance was less in the internal maxillary artery than the reported value for the mandibular arteries.

The respective vascular beds responded similarly to sympathomimetic and parasympathomimetic drugs.

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The Effect of Common Endodontal Antiseptics on Para-aminotoluene Sulfonamide (Benzylog)

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In a previous report it was shown that para-aminotoluene sulfonamide (PATS) is an effective and relatively non-toxic endodontal antiseptic.[1] Practitioners of endodontia, however, occasionally use two or more antiseptics in the course of root-canal treatment. It is both obvious and important that the behavior of para-aminotoluene sulfonamide be known when it is in the presence of other commonly used endodontal antiseptics.

This report concerns the in vitro measurement of antibacterial activity and change in activity of para-aminotoluene sulfonamide when it is exposed to various endodontal antiseptics routinely employed in root-canal therapy.

EXPERIMENTAL METHODS

Endodontal paper points previously impregnated with PATS as described elsewhere[1] were saturated with the secondary antiseptics and incubated therewith at 38° C. for 24 hours. Every reasonable effort was made to keep the points in a moist condition, yet free of any excess liquid. The secondary antiseptic was then either extracted, neutralized, or destroyed, the points dried at room temperature for 24 hours and then placed on a thioglycollate agar plate previously inoculated with a single loop of an endodontal culture which had been in incubation for 72 hours. Thioglycollate plates were incubated at 38° C. for 72 hours, after which the major axis, minor axis, and length of the paper point were measured and recorded for each zone of inhibition.

Since neutralization of the secondary antiseptic varied according to the antiseptic, it was necessary to conduct corresponding control tests on PATS-impregnated points to determine the effect of the neutralization procedure. In addition, plain endodontal paper points were impregnated with the secondary antiesptic only, subjected to the appropriate neutralizing procedure, and the respective antibacterial activities (0, if the neutralization procedure was adequate) determined. In certain instances it was necessary to conduct the neutralization procedure on plain paper points and to test these for antibacterial activity, since the procedure was of such a nature that it might have imparted an antibacterial effect to the paper points.

Thiosulphate test (reducing agent).—Four PATS-impregnated points were soaked in a small excess of 3 per cent cold sodium thiosulphate solution for 15 minutes, removed, placed on a sterile Petri dish, covered, and incubated at 38° C. for 24 hours.

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The points were maintained in a moist condition by occasionally adding small increments of the 3 per cent thiosulphate solution to each point.

After removal from the incubator, the paper points were allowed to cool and dry. Each point was then extracted with three 1-ml. portions of 70 per cent ethyl alcohol, dried at room temperature for 1 day, and placed on an inoculated agar plate as described earlier.

For purposes of comparison, the neutralization procedure was conducted on PATS-impregnated paper points, on plain paper points, and on thiosulphate-impregnated paper points.

Phenolics tests.—Phenol, eugenol, parachlorphenol, cresatin, cresote, and saponated cresol were the secondary antiseptics used in this portion of the study. The general procedure was the same as described in the thiosulphate test, except that the extracting agent was a mixture of diethyl ether and ethyl alcohol in the ratio of 1/1. Extractions were repeated until the ferric chloride test for phenolics gave a negative result.[2] The antibacterial activity of the points was determined as described earlier.

Chlorine compound tests (oxidizing agents).—Sodium hypochlorite (6 per cent active Cl_2), bis(2-hydroxy-5-chlorphenyl) methane* (7 per cent available Cl_2), and a saturated solution of the sodium salt of chlor-*p*-toluene sulfonamide† were used in this portion of the study.

After incubation, each impregnated point was treated with 0.1 ml. of 3 per cent potassium iodide solution. After liberation of iodine was complete, the points were placed individually in 0.1 ml. of cold 3 per cent sodium thiosulphate and 0.9 ml. of 95 per cent ethyl alcohol and agitated vigorously for 10 minutes. The liquor was decanted, and the extraction process repeated three more times. The points were then air-dried and tested.

Iodine tests (oxidizing agent).—Two per cent alcoholic iodine and 2 per cent Lugol's solution were used in this portion of the study. After incubation, each iodinated paper point was treated with thiosulphate and alcohol as just described above.

Silver nitrate test.—After incubation, the points were treated individually with 0.1-ml. volumes of ice-cold concentrated ammonium hydroxide. The extractions were repeated until they were free of detectable silver ion as shown by the addition of nitric acid and sodium chloride. The points were then extracted twice with cold 95 per cent alcohol, dried, and tested.

Hydrogen peroxide test (oxidizing agent).—A peroxidase solution was made by macerating 120 gm. of raw peeled potato and separating the solid and liquid phases by filtration. The clear filtrate was used immediately.

After incubation, the impregnated paper points were subjected singly to repeated extraction with 0.1-ml. quantities of cold peroxidase solution. The points were partly dried by extraction with 95 per cent alcohol, air-dried completely, and tested.

Quaternary base test.—Neutralization of the quaternary base, primarily cetyl-dimethyl-benzyl-ammonium chloride, was accomplished by treating the impregnated paper points with cold 2 per cent barium chloride solution, which inactivates the base by forming an insoluble salt.[3] Paper points impregnated with the quaternary base only were treated the same as the PATS experimental points, to test the adequacy of the neutralization procedure.

* Clorosene, or Chlorpactin. † Chloramine-T.

Formaldehyde tests.—PATS-impregnated points immersed in a 4 per cent formaldehyde solution form a white precipitate slowly. In a 40 per cent solution of formaldehyde the precipitate forms rapidly. The precipitate is very slightly soluble and forms readily upon mixing aqueous solutions of the two reagents. Since initial antibacterial testing gave negative results, no neutralization procedure was attempted.

RESULTS AND DISCUSSION

The character of the bacterial samples used in this study is shown in Table 1. Thioglycollate agar, inoculated with such bacteria provide an abundant growth on incubation. PATS-impregnated paper points, placed on such plates prior to incubation, produced well-defined zones of inhibition which were ellipsoidal in shape, with the dimension of its major axis highly dependent on the length of the paper point. Since the length of the paper point is not constant, direct comparison of two or more zones of inhibition may be misleading. To overcome this difficulty, an index rating has been devised and is defined as the product of the major and minor axes of the zone of inhibition divided by the length of the paper point producing the zone of inhibition ($d_1 \times d_2/L_p$). With this device it becomes possible to express a zone of inhibition as a simple whole number and to allow more accurate comparison of two or more such zones.

TABLE 1

GENERAL CHARACTER OF THE ENDODONTAL BACTERIAL SAMPLES USED IN THIS STUDY

Bacterial Culture	Gram Stain and Morphology
137	Gram-positive micrococci, numerous diplococci present
138	Gram-positive micrococci, primarily staphylococci
158	Gram-negative, short rods
159	Gram-negative and -positive mirococci

The results of all antibacterial tests expressed as index ratings are shown in Table 2.* Column 1 shows the ratings for PATS-impregnated paper points which have been treated with the respective secondary antiseptics, incubated, and the secondary antiseptic neutralized. Column 2 shows the index ratings of PATS-impregnated points which have undergone the neutralization procedure only, followed by antibacterial testing. It is to be expected that such procedure will probably diminish the activity of the PATS point in a measurable amount.

Column 3 ratings are a measure of the unaltered PATS points and serve as a reference standard. Column 4 shows the ratings of plain paper points impregnated with the secondary antiseptics, the antiseptics neutralized, and the resulting antibacterial activity measured accordingly. Such zero values lend assurance to the fact that PATS points so treated have little or no antibacterial activity present which may be credited to the secondary antiseptic. In those instances where the antibacterial activity of the secondary antiseptic was determined, the respective ratings are shown in Column 5. In some instances it was felt necessary to show that the neutralization procedure itself did not lend antibacterial potential to the PATS points. Ratings from such tests are shown in Column 6.

* Each value in the table is the average of four experimental determinations.

From Table 2, Column 3, it can be seen that against culture 137 PATS-impregnated points gave a control index rating of 24; against culture 158, a rating of 30; against culture 138, a rating of 31; and against culture 159, a rating of 14. It was necessary to adjust the data associated with cultures 158, 138, and 159 to the common majority control rating of 24, so that a more accurate comparison of the performance of the PATS points impregnated with the secondary antiseptics could be made. In the instance of para-monochlorphenol (PMCP) this was done as follows:

The ratio of the PATS control rating (Col. 3) to the PMCP index rating (Col. 1) is 30/26. When the control rating is reduced to 24, the PATS/PMCP ratio becomes

TABLE 2*

COMPARATIVE ACTIVITY OF PATS-IMPREGNATED PAPER POINTS AFTER TREATMENT WITH RESPECTIVE SECONDARY ANTISEPTICS

Bacterial Culture	Secondary Antiseptic	Index Ratings†					
(1)	(2)	PSN (3)	PN (4)	P (5)	SN (6)	S (7)	N (8)
137.....	Sodium thiosulphate, 3%	19	19	24	0	0	0
137.....	Iodine, 2%, alcoholic	25	22	24	0	—	—
137.....	Lugol's solution, 2%	24	19	24	0	—	—
137.....	Ethyl alcohol, 95%	23	19	24	0	0	—
137.....	Ethyl alcohol, 70%	24	19	24	0	0	—
137.....	Phenol, 95%	23	19	24	0	—	—
137.....	Saponated cresol	25	22	24	0	—	0
158.....	Para-chlorphenol	26	23	30	0	61	—
158.....	Eugenol	25	26	30	0	36	0
138.....	Cresatin	27	29	31	0	2	0
138.....	Cresote	29	29	31	0	—	0
137.....	Sodium hypochlorite	19	19	24	0	—	0
137.....	Chlorosene	15	19	24	0	—	0
137.....	Silver nitrate, 2%	0	18	24	0	—	0
159.....	Chloramine-T	14	17	14	0	0	—
138.....	Hydrogen peroxide, 30%	26	24	31	4	16	—
158.....	Quaternary base, 4%	13	14	30	3	6	0
158.....	Formaldehyde, 40%	0	—	30	—	—	—

* See text for explanation.

† Code: P = PATS-impregnated paper point, unaltered; S = Secondary antiseptic treatment added; N = Neutralization procedure applied; — = no determination made.

24/21. In this instance the neutralization procedure caused a loss of 7/30 of the initial rating (Col. 3 — Col. 2). On the basis of a control value of 24, the correction factor for this loss becomes +5. The adjusted value of the PATS/PMCP index rating becomes 26 (21 + 5).*

From the data compiled on the basis of a control index rating of 24, the bar graph of Figure 1 was constructed. It is clear that for practical purposes, the majority of the agents tested had little effect on the antibacterial activity of the PATS-impregnated points, whereas it is equally clear that silver nitrate and formaldehyde were completely incompatible with PATS. This result occurred because a reaction between PATS and the agents formed an insoluble, inactive precipitate.

* It is a coincidence that the adjusted value and the initial value were identical.

SUMMARY

Seventeen agents, most of which may occasionally be used in root-canal therapy, were investigated with regard to their effect on the antibacterial activity of PATS-impregnated paper points.

A majority of these agents were compatible with PATS. Silver nitrate and formaldehyde completely destroyed the activity of PATS.

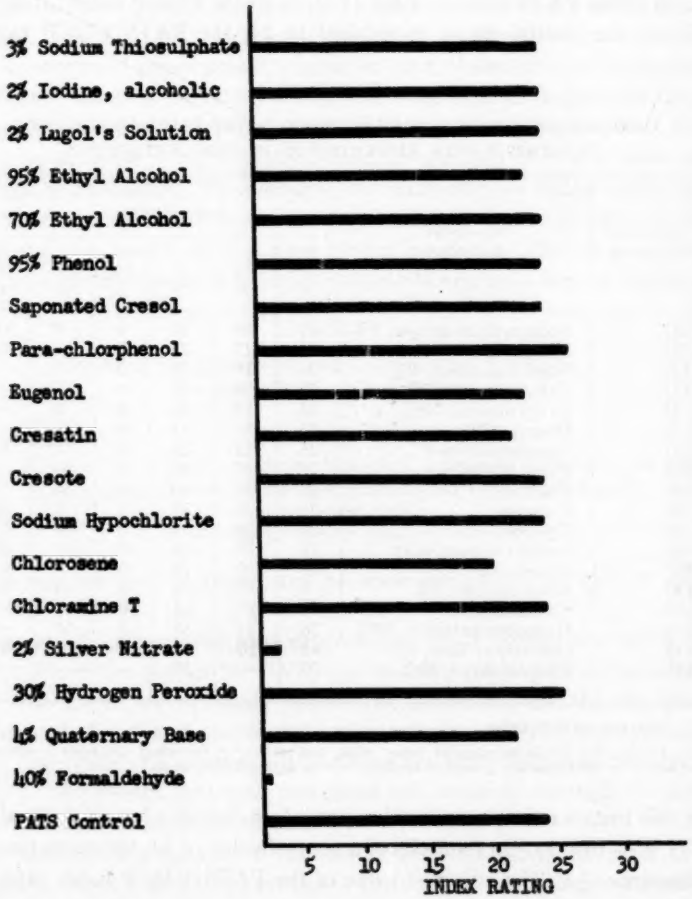

FIG. 1.—In vitro antibacterial activity of PATS-impregnated endodontal paper points following contact with other endodontal antiseptics. See text for explanation.

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Concomitant Calculus and Caries

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While obtaining dental calculus samples from producers for subsequent chemical studies on calculus formation, it was observed that calculus producers of all ages exhibited varying degrees of caries activity. In any studies on factors involved in dental calculus formation the possible antagonism of the caries and calculus processes would have to be considered. Indeed, Hodge and Leung[1] suggest that the physicochemical conditions in saliva which contribute to enamel decalcification (caries?) inhibit calculus formation. Bibby[2] likewise concluded from an extensive review of the literature that "calculus deposition and increased putrification associated with periodontal pathology oppose caries and variations in salivary chemistry found in periodontitis occur with low caries activity." On the other hand, Maldanado,[3] Tishler,[4] and Miller and Seidler[5] present clinical findings that caries occurrence is independent of periodontal disease. This study was undertaken to clarify the relationship, if any, between increased calculus deposition and caries activity in calculus producers, in order to design subsequent experiments on factors which may predispose to calculus.

EXPERIMENTAL METHODS

Out of 1,144 adult and child patients appearing in the prophylaxis clinic of the Eastman Dental Dispensary, Rochester, New York, who were examined by one of the authors for hard deposits, 295 subjects were confirmed by us as calculus producers. The sex, socio-economic status, and age at last birthday of those showing the deposits were recorded, as well as the caries experience, gingival conditions, and the type and amounts of all calculus-like deposits. The calculus deposits were carefully removed either by the examining dentist (H. S. W.) in the case of subgingival deposits or by the hygienists in the other areas. The calculus was collected in individual sterile bottles marked with the tooth location.

I. EVALUATION OF CALCULUS

Clinical.—The location and amount of calculus were charted for each subject. Calculus was regarded as supragingival if it was entirely above the gingival margin, as subgingival if it was completely below the gingival margin, and mixed if deposits were present in both areas. The amounts of calculus were graded after the method of Davies.[6] *Supragingival* calculus was considered to be "slight" if thin deposits were con-

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fined to the clinical crowns of the lower anterior teeth or the buccal of the upper posterior teeth in the vicinity of the gingival margin; "moderate" if deposits covered most of the coronal surfaces of the lower six anterior teeth or upper posterior teeth; and "severe" if heavy deposits covered most of the coronal surface of more than the six lower anterior teeth. *Subgingival* calculus was considered as "slight" if thin deposits were confined to the lower six anterior teeth or upper posterior teeth; "moderate" if thin deposits were present on more than six anterior teeth or upper posterior teeth; and "severe" if heavy deposits were present on more than the six anterior teeth. In cases of mixed calculus, the amount and location of supragingival as well as subgingival calculus was charted.

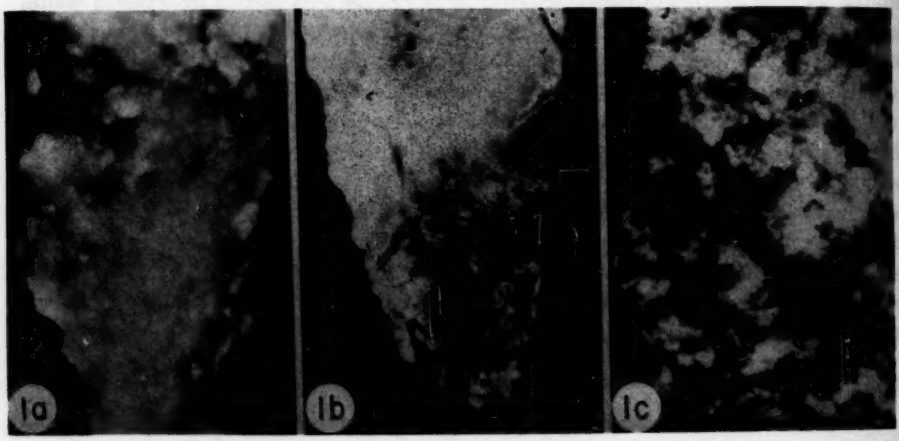

FIG. 1.—*a*, Photomicrograph of Type 1 dental calculus using incident light. (Orig. mag. ×110.) *b*, Microradiograph of Type 1 dental calculus exposed at one and one-third standard exposure time. (Standard exposure time = exposure time necessary to produce a maximum photographic density of 2.2 in a similar thickness of dentin.) (Orig. mag. ×100.) *c*, Microradiograph of Type 2 dental calculus at standard exposure time. This is representative of the minimum degree of calcification accepted as calculus in this study. Note the spongelike pattern in this type. (Orig. mag. ×100.)

Laboratory.—To confirm the clinical diagnosis of calculus, the samples collected from each patient were studied in the laboratory, using a soft X-ray technique. Pieces or sections of the calculus, 100–400 μ thick, were exposed on spectroscopic plates* at a distance of 10 cm. to the characteristic Kα radiation (1.54 A) of a Cu target mounted in a Picker machine.† The Machlett tube was run at 20 kv. and 10 ma. for time intervals sufficient to penetrate and expose a similar thickness of normal dentin as established by one of the authors in previous work.[7] The outline of normal deposits was semiradio-opaque but well defined. It was contiguous with a radiolucent network throughout the sample. In some samples the network and non-network were almost entirely radio-opaque (Type 1, Fig. 1, *b*). In many cases increasing the exposure to that needed for enamel was necessary to obtain any detail. In other samples a spongelike appearance was evident, with the network much wider, radiolucent, and fibrous in appearance (Type 2, Fig. 1, *c*). Cross-sections of both extremes showed radiolucent

* Kodak #649-0.

† Picker X-Ray Corp., Division Waite Mfg. Co., Cleveland, Ohio.

incremental lines (Fig. 2, *a* and *b*). On higher magnification, interconnections are seen between the radio-opaque masses and the more radiolucent incremental lines (Fig. 2, *c* and *d*). Fissures of extreme radiolucency were a common characteristic of the more radiolucent pieces. Lightly calcified deposits clinically were of two types—one of a soft gritty nature and the other hard, with a definite shape which decreased markedly in volume on removal from the mouth. These were almost completely radiolucent under the above exposure conditions, as was materia alba secured from both calculus producers and non-producers. Both lightly calcified concretions and materia alba showed a diffuse slight radio-opacity at shorter exposure times. Many distinct, somewhat dis-

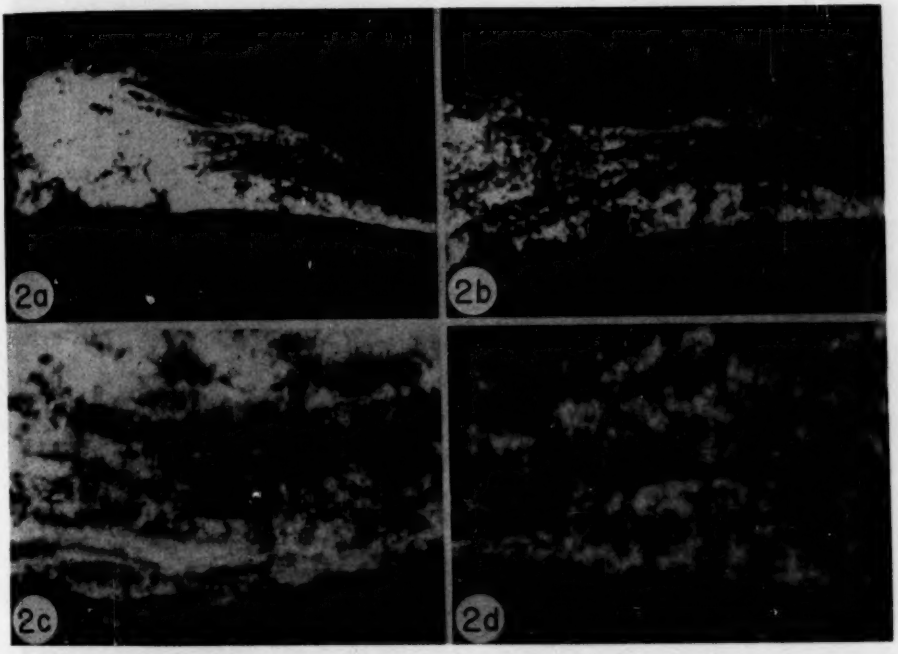

FIG. 2.—*a* and *b*, Microradiographs of cross-sections of calculus Type 1 and Type 2, respectively. Note the radiolucent incremental lines. (Orig. mag. ×56.) *c* and *d*, Higher magnification of the above sections. Note the interconnections between the radio-opaque masses and the radiolucent incremental lines. (Orig. mag. ×240.)

connected, areas of high radio-opacity appeared in soft deposits (Fig. 3, *a* and *b*), whereas materia alba had only a few (Fig. 4, *a* and *b*). Only subjects with concretions showing radio-opacities falling within the range of Types 1 and 2 (Figs. 1 and 2) were accepted as calculus producers in this study.

II. CARIES EVALUATION

Clinical.—Caries evaluation was done by the same investigator at the time that observations on calculus were being made. Explorers and mouth mirrors, as well as bite-wing X-rays, were used for diagnosis. Observations were made on the state of the carious lesions,[8] and, for the purpose of classifying these patients as to caries activity, the following groups were recognized: (1) "active caries," in which all carious lesions

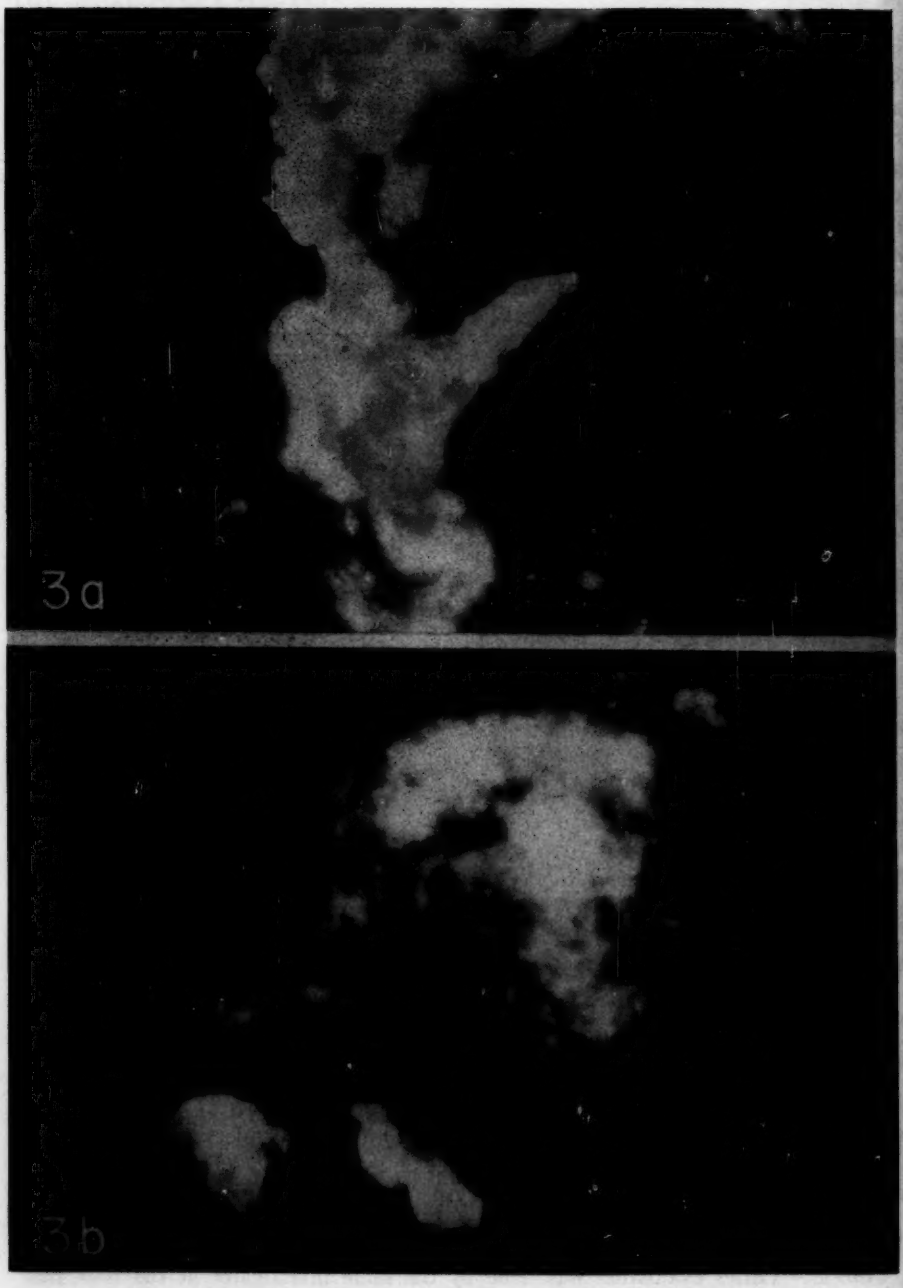

FIG. 3.—*a*, Photomicrograph of an unacceptable deposit, clinically of a soft, gritty nature. (Orig. mag. ×115.) *b*, Microradiograph of this deposit showing limited areas of high radio-opacity at one-third the standard exposure time. (Orig. mag. ×100.)

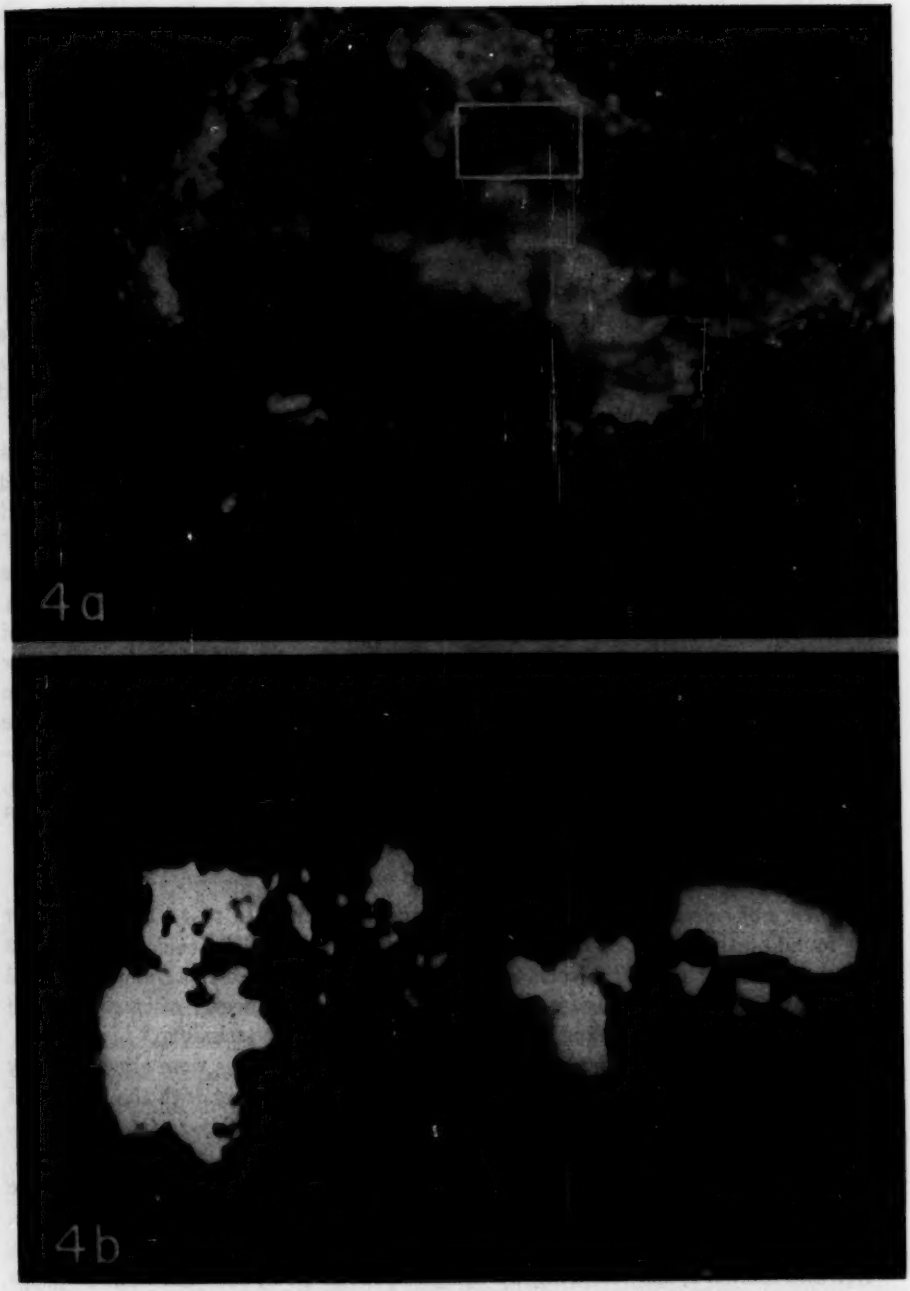

FIG. 4.—*a*, Photomicrograph of materia alba. The area within the box was the only portion where calcification was found. (Orig. mag. ×115.) *b*, Enlargement of the microradiograph of the only area in the above materia alba sample which showed discrete areas of radio-opacity at one-third standard exposure time. (Orig. mag. ×540.)

were soft and spongy to the probe or on the inaccessible proximal surfaces there was definite penetration into the dentin as shown in the bite-wing X-rays; (2) "inactive caries," in which all carious lesions were hard and glossy or did not penetrate beyond the dentino-enamel junction in the bite-wing X-rays (recurrent decay fell into this classification);* (3) "active-inactive caries," in which there were both active and inactive lesions according to the previous definitions; (4) "restored caries," in which all lesions were restored with no evidence of new caries; and (5) "caries-free," in which cases there were no lesions and no fillings.

The use of such broad terminology as "active" caries may be questioned. However, the point under examination is whether the caries, which clinically could be called a progressing lesion, would be found along with calculus. To test the validity of this assumption, as many as possible (42) of the heaviest calculus producers were placed on 4-month recall following the removal of all calculus.

The above classification of caries was based on previous observations on non-calculus producers:[8] most cavities having a low resting pH (mean 5.6 ± 1.2) were soft and spongy with thick layers of decay ("active" caries), while cavities with a higher pH (mean $6.7 \pm .7$) were generally hard and glossy with thin layers of decay ("inactive" caries). Hence if pH is an indication of caries activity, the pH of cavities described as "active" in calculus and non-calculus producers should be equally low and vice versa.

Laboratory.—To substantiate the clinical evaluation of the caries, the initial pH of carious lesions and acid production in them was measured before and after a glucose rinse. For this purpose a pH meter† was used, employing a microantimony electrode in conjunction with a calomel electrode and saturated KCl salt bridge according to the method previously described by Caldwell and Bibby.[9] After recording initial pH's, subjects rinsed their mouths with 20 cc. of a 10 per cent glucose solution for 30 seconds, and pH measurements were made in the lesion at intervals of 1, 3, 5, 10, 15, 20, 25, and 30 minutes after the rinse.

RESULTS

Incidence of calculus.—Calculus deposits ranging from 0.015 to 4.00 gm. in weight were found on the teeth of 295 of the 1,144 subjects examined for calculus only (25.8 per cent). Fifteen other subjects, although clinically acceptable, were not included among calculus producers because of their having a too low degree of calcification of the deposits as determined experimentally by the soft X-ray technique.[9]

Sex distribution of the calculus producers.—Males and females were almost equally affected by calculus. The results as measured here were consistent with the hypothesis that there was no difference between calculus incidence in males and females (chi-squared $= 0.974$ with a probability greater than 30 per cent).[10]

Socio-economic distribution.—One hundred and ninety-eight (67 per cent) of the 295 subjects affected with calculus fell into the middle and upper socio-economic group, as based on residence, income, and occupation of the wage earner.

Age distribution of subjects with supragingival, subgingival, and mixed calculus.—In Table 1, the distribution by age of both types of supragingival calculus is shown. It is

* It is recognized that active, early carious lesions would involve only the enamel and thus fall into this classification.

† Beckman Model H-2.

apparent that the greater number of subjects had either supragingival or mixed calculus (91.8 per cent) and, moreover, that supragingival calculus (both mixed and alone) occurred in almost equal frequency in every age group of calculus producers. With increasing age, however, there was a significant tendency toward mixed calculus formation rather than supragingival alone. Since the results did not agree with the hypothesis that there was no difference between mixed calculus and age (chi-squared = 26.29 with a p of < 1 per cent), the alternative hypothesis was embraced. However, the forty to forty-nine age group had a greater percentage of people with supragingival alone, possibly indicating incipient calculus formation in that group.

In spite of the fact that the proportion of people with supragingival calculus alone decreased with increasing age, the distribution within each age group of slight,

TABLE 1

DEGREE OF SUPRAGINGIVAL CALCULUS FORMATION BY AGE

Age at Last Birthday	Total No. Subjects	Supragingival Calculus Alone							Supragingival Calculus (Alone or Mixed)						
		Per Cent of All Affected	Degree of Calculus Formation						Per Cent of All Affected	Degree of Calculus Formation					
			Slight		Moderate		Severe			Slight		Moderate		Severe	
			No.	Per Cent	No.	Per Cent	No.	Per Cent		No.	Per Cent	No.	Per Cent	No.	Per Cent
0– 9.....	8	75.0	3	50.0	1	16.7	2	33.3	87.5	4	57.1	1	14.3	2	28.6
10–19.....	72	66.7	31	64.6	10	20.8	7	14.6	86.1	36	58.1	18	29.0	8	12.9
20–29.....	74	48.6	19	52.8	15	41.7	2	5.6	90.5	25	37.3	29	43.3	13	19.4
30–39.....	76	56.6	19	44.2	18	41.9	6	14.0	97.4	22	29.7	30	40.5	22	29.7
40–49.....	39	71.8	16	57.1	8	28.6	4	14.3	97.4	18	47.4	14	36.8	6	15.8
50+.....	26	34.6	4	44.4	2	22.2	3	33.3	88.5	8	34.8	3	13.0	12	52.2
Total.	295	57.6	92	54.1	54	31.8	24	14.1	91.9	113	41.7	95	35.1	63	23.2

moderate, or severe amounts did not change. The results were consistent with the hypothesis that there was no relationship between age and supragingival calculus severity as measured here (chi-squared = 13.0 with a $p > 0.30$).

In contrast to people with supragingival alone, the amount of deposits in people with both supragingival calculus alone and mixed was found to increase with the age of the subjects, the difference being related to increased severity of the supragingival calculus in the mixed group. Chi-squared on the basis of no difference was 28.27, a value which will be exceeded by chance less than 1 per cent of the time, and was found to support the alternative hypothesis.

The findings related to the age of subjects with subgingival calculus are given in Table 2. The percentage of calculus producers affected by subgingival calculus alone did not change with age. The proportion of subjects affected by mixed subgingival calculus, however, did increase with age. Moreover, on the basis of no relationship between age and severity, chi-squared was 18.93, with a probability of < 0.05, indicating

that there was a trend toward deposition of increasing amounts of subgingival calculus with increasing age.

Caries experience of calculus producers.—Table 3 shows the age distribution of the caries activity among the calculus producers. At least 69 per cent of every age group was affected with caries. However, after forty years of age there was a significant decrease in the proportion of subjects having "active" caries.

Contrary to expectations, Table 4 shows that there was no change in caries experience with increasing deposition of either supra- or subgingival calculus. A similar caries

TABLE 2

AMOUNT OF SUBGINGIVAL CALCULUS FORMATION BY AGE

Age at Last Birthday	Total No. Subjects	Subgingival Only		Mixed		All Subgingival Degree of Calculus Formation					
						Slight		Moderate		Severe	
		No.	Per Cent of All Affected	No.	Per Cent of All Affected	No.	Per Cent	No.	Per Cent	No.	Per Cent
0– 9	8	1	12.5	1	12.5	2	100.0	.....	.....	.....	.....
10–19	72	10	13.9	14	19.4	15	62.5	4	16.7	5	20.8
20–29	74	7	9.5	31	41.9	13	34.2	10	26.3	15	39.5
30–39	76	2	2.6	31	40.8	8	24.2	9	27.3	16	48.5
40–49	39	1	2.6	10	25.6	2	18.2	3	27.3	6	54.5
50+	26	3	11.5	14	53.8	4	23.5	2	11.8	11	64.7
Total	295	24	8.1	101	34.2	44	35.2	28	22.4	53	42.4

TABLE 3

CARIES OCCURRENCE IN CALCULUS PRODUCERS BY AGE GROUP

Age at Last Birthday	No. Subjects	Number and Per Cent of Subjects									
		With Caries							Without Caries		
		Active Caries		Active and Inactive		Inactive		Per Cent with Carious Lesions	No. Affected		Per Cent with No Lesions
		No.	Per Cent	No.	Per Cent	No.	Per Cent		Restored	Free	
0– 9	8	7	87.5	.....	.....	.....	.....	87.5	.....	1	12.5
10–19	71*	56	78.9	5	7.0	4	5.6	91.5	3	3	8.5
20–29	74	52	70.3	11	14.9	4	5.4	90.5	7	.....	9.5
30–39	76	46	60.5	11	14.5	10	13.2	88.2	9	.....	11.8
40–49	38*	17	44.7	4	10.5	7	18.4	73.7	7	3	26.3
50+	26	8	30.8	1	3.9	9	34.6	69.2	8	.....	30.8
Total	293	186	63.48	32	10.9	34	11.60	86.0	34	7	14.0

* X-rays on 1 patient in group questionable.

distribution was found whether the deposits were slight, moderate, or heavy and supragingival or subgingival. In fact, of those subjects with supragingival calculus, 89 (38.5 per cent) had calculus deposits on at least one carious tooth. Their age distribution is shown in Table 5, together with the assessed activity of the carious lesions. Approximately an equal percentage of subjects in every age group had calculus on carious teeth. Moreover, by our criteria, 87 per cent of the lesions were "active."

The caries experience of the 47 subjects who were abnormally heavy supragingival

TABLE 4

AMOUNT OF CALCULUS FORMATION AND CARIES ACTIVITY

AMOUNT OF CALCULUS	No. SUBJECTS	NUMBER AND PER CENT OF PATIENTS							
		With Caries						Without Caries	
		All Active		Some Active, Some Inactive		All Inactive		Restored or Free	
		No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent
Supragingival:									
Slight	113	69	61.1	9	8.0	14	12.4	21	18.6
Moderate	93	63	67.7	15	16.1	6	6.5	9	9.7
Severe	63	38	60.3	6	9.5	11	17.5	8	12.7
Total	269	170	63.2	30	11.2	31	11.5	38	14.1
Subgingival:									
Slight	43	27	62.8	8	18.6	5	11.6	3	7.0
Moderate	28	20	71.4	2	7.1	3	10.7	3	10.7
Severe	53	33	62.3	8	15.1	6	11.3	6	11.3
Total	124	80	64.5	18	14.5	14	11.3	12	9.7

TABLE 5

CARIES ACTIVITY IN TEETH WITH SUPRAGINGIVAL CALCULUS ON SAME TOOTH

AGE AT LAST BIRTHDAY	No. SUBJECTS	NUMBER AND PERCENT OF SUBJECTS WITH				PER CENT OF THESE TEETH THAT WERE ACTIVE
		Calculus on the Carious Tooth		Active Caries		
		No.	Per Cent	No.	Per Cent	
0– 9	8	2	25.0	2	25.0	100
10–19	72	19	26.4	19	26.4	100
20–29	74	24	32.4	22	29.7	91.7
30–39	76	22	29.0	19	25.0	86.4
40–49	39	10	25.6	9	23.1	90.0
50+	26	12	46.2	7	26.9	58.3
Total	295	89	30.17	78	26.4	87.64

calculus producers showed that heavy calculus producers were found in almost equal proportions in every age group and, furthermore, that at least 69 per cent of these subjects had "active" caries. On examination at the first 4-month interval, most of the "active" lesions had progressed, as shown by X-ray, and new calculus had deposited. At the 8-month interval, either new lesions were discernible, or recurrent decay had developed together with additional calculus.

When the pH of cavities and the drop in pH after a glucose rinse in mouths of calculus and non-calculus producers were compared, the curves were found to be essentially

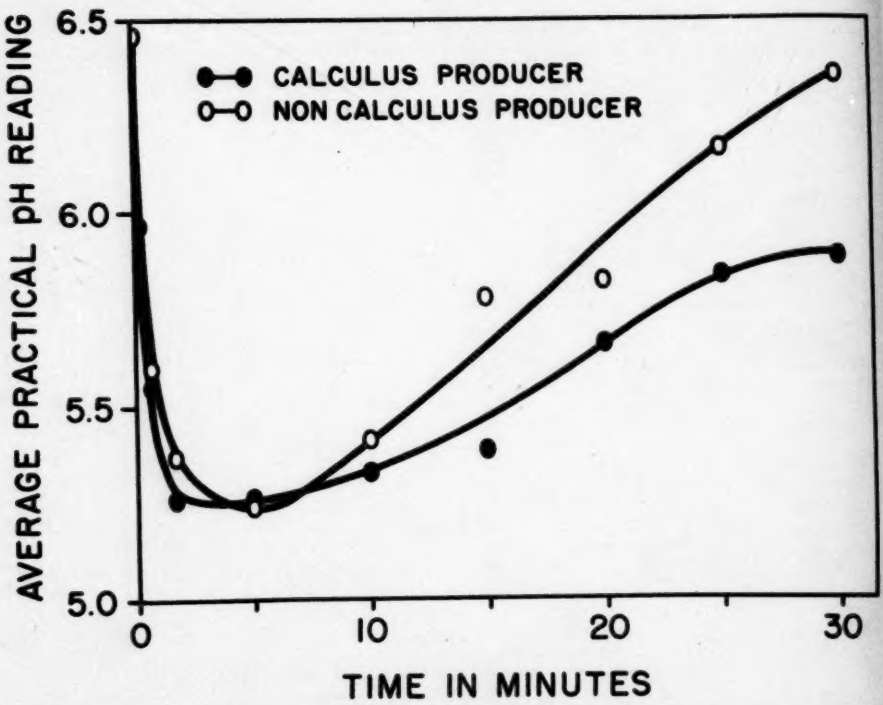

FIG. 5.—The average of practical pH number (Bates, 1954) in cavities of calculus and non-calculus producers before and after a 30-second, 10 per cent glucose rinse over an interval of 30 minutes. The mean deviation of the initial readings was 1.1 pH units, and that of all others 0.6-0.8 pH units. The fall and rise in pH in cavities of calculus and non-calculus producers were similar.

the same. The average of 28 tests in cavities in calculus producers and 41 tests in non-producers is given in Figure 5. The fall and subsequent rise in pH following a glucose rinse is similar to, and follows, that reported by Caldwell and Bibby[9] and others.[11] Furthermore, the pH in these lesions did not vary significantly from that in lesions of the same description in non-calculus producers.[9]

DISCUSSION

This study failed to find any relationship (direct or indirect) between the amount of dental calculus deposition and caries activity in calculus producers. Caries commonly accompanied supragingival, subgingival, or mixed calculus and was commonly found in

a calculus-incased tooth. In fact, progressing lesions along with continued calculus deposition were observed in many of the heaviest calculus producers from every age group. Moreover, when the caries in calculus producers was subjected to glucose, the fall in pH was similar to that found previously in caries of nonproducers.[8]

Although a decrease in caries activity and an increase in the amount of mixed calculus deposition was noted with increasing age, no correlation was found between the two conditions either for the whole group or in any specific age group. This suggests that, in calculus producers as well as in the general population,[12] caries activity decreases with age and is independent of the influence of increasing supra- or subgingival calculus deposition. Therefore, there must be conditions in the mouths of calculus producers which lead to caries and a different set of conditions which control the deposition of calculus. Further studies are obviously required to elucidate the underlying factors which predispose to either of these two conditions.

SUMMARY

As a preliminary to a detailed laboratory study of calculus, 1,144 consecutive patients ranging in age from five to eighty were examined for dental calculus in the Eastman Dental Dispensary Prophylaxis Clinic by a dentist. Of these, 295 subjects (25 per cent) had deposits confirmed as calculus in the laboratory by radio-opacity to soft X-ray. Clinically, 91 per cent of the calculus subjects had supragingival deposits. Of these, 34.6 per cent had subgingival deposits either combined with the supragingival or on other teeth. The proportion of calculus subjects with mixed calculus increased significantly with the age of the subject. The occurrence of slight, moderate, or severe amounts in those subjects with supragingival calculus alone did not vary with age, but the proportion of subjects in the groups decreased.

However, the proportion of subjects and amount of supragingival deposits increased significantly with age when subjects with both mixed and supragingival calculus were considered.

A simple classification of the caries showed that, among calculus subjects, at least 69 per cent of each age group was affected by active caries, but the proportion affected after forty years of age decreased significantly. However, increasing amounts of either supra- or subgingival calculus had no effect on caries activity of the calculus producers. In fact, 89 people (30.3 per cent) had supragingival calculus on carious teeth, 87 per cent of which fell in the classification of active lesions. Of the abnormally heavy calculus producers, 69 per cent had active lesions. The lesions of calculus producers cited and another group of non-producers showed a similar pH response to a glucose rinse.

The authors wish to commend the second-year students of the Eastman Dental Hygienists School for their superb co-operation in the collection of samples under the direction of Dr. Ruth Vann and Miss Florence Arlidge, D. H.; to thank Dr. Basil G. Bibby and Dr. Roland Hawes for their helpful suggestions in the organization and pursuit of this study; and to recognize the valuable technical assistance of Cosamine Casciani.

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An Analysis of the Sounds Produced by the Mandibular Joint

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Clicking of the mandibular joint has been recorded as an important symptom and sign of temporomandibular arthrosis. Ireland,[1] in particular, emphasized the importance of crepitus and indicated clinical divisions of symptoms and signs which were related to this factor. Hankey,[2, 3] however, in a study of over 200 patients found that crepitus occurred in only 57 per cent, and in about half of these there was associated pain. Anatomically, the "click" has been explained by Rees[4] as the result of the condylar ridge slipping over the anterior or posterior transverse band of the meniscus.

The use of the stethoscope is thus reasonable in the diagnosis of mandibular joint conditions,[5] and it follows that permanent records of joint sounds on electromagnetic tape, on photographic mediums, or with ink pens on paper provide data for a qualitative and quantitative evaluation of crepitus. Ekensten[6] described such an investigation. A constant-amplitude crystal microphone was held by the subject over the joint area, and after amplification the unilateral joint sounds were recorded photographically. Ekensten called these records "phonograms." In a qualitative report relating these records to clinical and radiographic findings, he concluded that the normal joint was silent and suggested great possibilities for this form of record.

The present work embodies an approach similar to that of Ekensten. The sound record of joint noise was termed the "phonoarthrogram" (P.A.G.) because it related the technique used in the present investigation to the phonocardiogram[7] and the phonomyogram.[8] However, it was realized during the initial phases of this work that interpretation of the phonoarthrogram along the lines of a phonocardiogram as a regular, well-defined pattern was not possible. It was therefore decided to investigate quantitatively only two aspects: the over-all pattern of joint sounds and the difference, if any, between normals and abnormals. Qualitative observations are also presented as a guide in the evaluation of variables that might influence the quantitative results.

EXPERIMENTAL METHODS

Bilateral simultaneous records of joint sounds and unilateral records of joint sounds in conjunction with records of jaw movement were taken consecutively on 200 individuals. From this series, twenty cases (10 normal and 10 abnormal) were chosen, irrespective of sex, age, occlusal, or skeletal pattern, etc., for the investigation of unilateral records. The bilateral records of a further 48 cases, 24 of each type, normal or abnormal, comprising 12 females and 12 males each, were examined, irrespective of other factors.

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The abnormals all had temporomandibular arthrosis, for which some were undergoing treatment. The diagnosis and treatment of these cases was under the direction of Mr. G. T. Hankey, of the London Hospital. The normals were students or staff of the London Hospital.

Joint sounds were recorded by means of a constant-velocity, transducer microphone (Fig. 1).* Designed primarily as a bone-conduction hearing aid, this microphone is contoured to fit the mastoid bone and was found to be equally suitable for recording

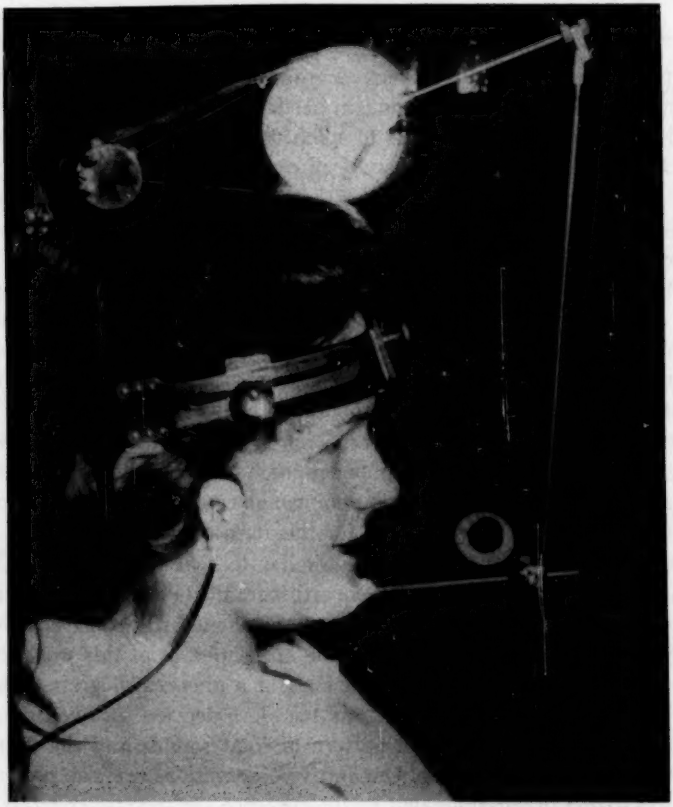

FIG. 1.—Right-side microphone and jaw-movement apparatus in position on patient

sounds from the mandibular joint region. The dimensions of the microphone are approximately 28 by 20 mm., and its positioning in each case was directly over the mid-point of the outer rim of the articular fossa, approximately 10 mm. lying above and 10 mm. over the zygomatico-temporal arch. The posterior margin of the microphone rests against the tragus and is maintained in contact with the tissues by means of a steel head band.

The recordings were made in a large room with the windows closed to avoid direct noise. A full investigation was made of the possible interference noises or movements, and it was found that the pickup range of the microphone, when in position on the head,

* Fortiphone Ltd., London.

was remarkably unidirectional. Skull conduction of joint noise from one side to the other occurred but was easily detectable by the exactness of its duplication and its lower amplitude.

A comparison of recordings from the frontal, mastoid, and zygomatico-temporal regions indicated an identical over-all pattern, but with the maximum noise recorded directly over the joint. Movements of the microphone itself due to condylar motion beneath it were minimum.

Sounds were amplified by two pre-amplifiers,* at the d.c. setting, coupled to the high-amplification sides of two twin-beam oscilloscopes.† Both groups of amplifiers were placed at maximum gain so as to give an amplification of 45,000 on both channels of one oscilloscope screen.

For recording jaw movement the main amplifier of one beam was placed at the 100-volt position. All leads were screened.

The oscilloscopic trace was photographed by a fixed-focus camera,‡ with an end-of-film indicator. The recording speed was kept constant at 2.5 inches per second. Recording paper§ was used, and developing, fixing, and washing techniques were kept standard throughout the investigation.

A lighted open-close instructor was used to initiate and control patients' jaw movements to avoid possible pickup by microphones of the operator's voice. The instructor consisted of a partitioned box containing two 60-watt tungsten tube lights‖ which illuminated the words "open" and "close." These lights were controlled by the operator by means of a silent two-way switch.‖

The open-close jaw movement was recorded by attenuating the 50-cycle calibration signal provided by the main amplifier with a low-torque potentiometer. The apparatus consists of a perspex plate arranged sagittally on the head like a cock's comb and retained in position by a horizontal head band (Fig. 1). The plate carried the potentiometer posteriorly and a wheel anteriorly with a projecting rod attached to its axle. A belt drive from the rim of the wheel to the axle of the potentiometer gave a full electrical deflection for 25° of movement. The projecting rod of the wheel was connected by a universal joint to a vertical arm which hung down perpendicularly and centrally, approximately 5 cm. in front of the face. The vertical arm joined an adjustable horizontal arm which was strapped to the subjects' chin with zinc oxide tape. Mainly because of the tissue movement, this apparatus had an error varying from 3 to 10 mm. However, it provided a definite method of relating joint noise to mouth opening.

In this paper the opening and closing phases in unilateral records were defined as the interval between the first sound and the first point of maximum deflection of the jaw-movement trace and, for the closing phase, the interval from the last point of maximum deflection to the last sound. The neutral phase was therefore the interval of maximum deflection.

The sequence of recording, after several trial open-close movements, was as follows: (1) sound only, left and right joints simultaneously, bilateral record; (2) sound from left joint coupled with jaw-movement signal, unilateral record; (3) sound from right

* Cossor 1434, A. C. Cossor Ltd., London.

† Cossor 1049, A. C. Cossor Ltd., London.

‡ Cossor 1428, A. C. Cossor Ltd., London.

§ Kodak R.P. 30, Kodak Ltd., London.

‖ General Electric Co., London.

joint coupled with jaw-movement signal, unilateral record. The open-close movement was repeated a minimum of three times in each of 1, 2, and 3 (Fig. 2).

RESULTS

Interpretation of sound records.—Three mechanical factors must be considered during the interpretation of each record: (1) the range and control of the movement, (2) the degree of intracapsular inflammation, and (3) the pattern and velocity of the occlusal contact.

At the amplification used in the present study, all normal joints showed some noise in the opening and closing phases (Fig. 2).

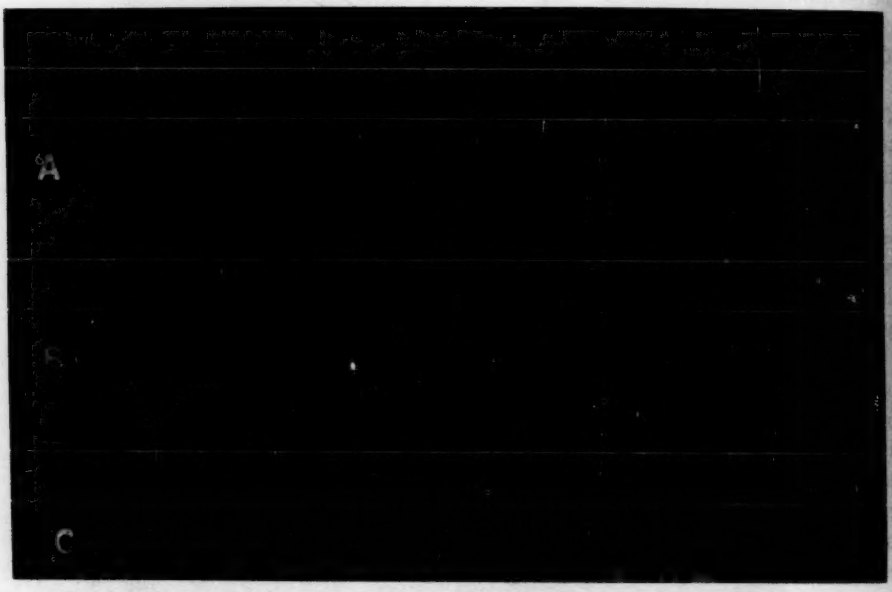

FIG. 2.—Normal case. Recordings for reproduction were made on 35-mm. film to obviate the halation effect achieved by reproduction of recordings made on recording paper. *A*—upper channel, left joint; lower channel, right joint. *B*—upper channel, left joint; lower channel, jaw-movement registration. *C*—upper channel, right joint; lower channel, jaw-movement registration.

Two main types of sound were differentiated: the click, a single diphasic sound (Fig. 3); and multiple diphasic and polyphasic sounds, i.e., clicking, cracking, and grinding noises.

The sounds observed during the opening and closing phases appeared to be related to the anatomical explanation of the relationship of the condylar ridge to the posterior and anterior portions of the disc.

Integration and analysis of sound records.—The traces of ten normal and ten abnormal subjects were measured. To summarize the unilateral records, each trace was divided into an opening, neutral, and closing phase by reference to the jaw-movement trace. The first and last phases were bisected, giving five phases in all, denoted by O_1, O_2, N, C_1, C_2. The maximum signal height in each phase was measured to the near-

est millimeter and tabulated. No allowance was made for variable base height or duration. The maximum signal height proved to be non-normally distributed, with means proportional to standard deviations in subgroups. Since the analysis of variance requires the assumption that the variate be normally distributed with equal subgroup variances, it was necessary to transform the variate. Conversion of the maximum signal height to logarithms to the base e proved satisfactory.

A summary of the analysis of variance is shown in Table 1. At the conventional 5 per cent level of significance ($P = 0.05$), only phase and subject effects were significant, P being less than 1 per cent ($P < 0.01$). The five-phase means of these twenty subjects are tabulated from lowest (N) to highest (C_2) in Table 2, in logarithms, and

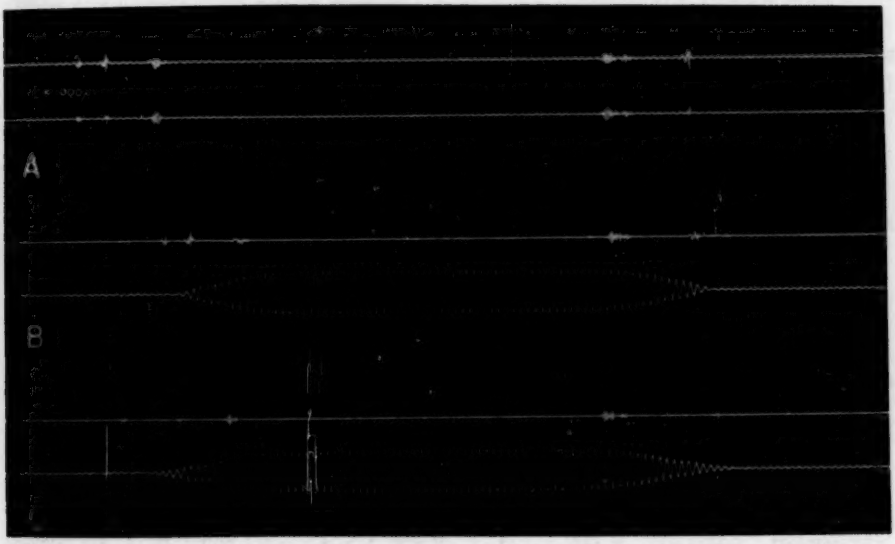

FIG. 3.—Abnormal case. Note "click" in left joint on opening. *A*.—upper channel, left joint; lower channel, right joint. *B*—upper channel, left joint; lower channel, jaw-movement registration. *C*—upper channel, right joint; lower channel, jaw-movement registration.

the equivalent reading in millimeters. Application of the "new multiple range test"[9] showed the neutral-phase mean (N) to be significantly lower than the mean of any other phase. This was as expected, since the neutral phase should be silent, apart from recording errors. Comparable halves of the opening and closing phases (i.e., O_1, C_2 and O_2, C_1) were not significantly different. P.A.G. records of condylar movement would therefore appear to be symmetrical except for the significant increase from the first to the second half of the closing phase, i.e., $\overline{C}_1 < \overline{C}_2$. This is not matched in the opening phase where O_1 and O_2 are not significantly different (Fig. 4). No significant differences in this pattern were detected between normal and abnormal types or between the separate records of right and left sides.

Although there were significant differences between the 20 subjects in the production of recorded noise, this was not due to abnormality, since no significant differences were observed between the means of normal and abnormal types. Since the sides effect in this

analysis approached significance (Table 1) and because the right and left sides were recorded in sequence, further investigation using simultaneous bilateral records was considered necessary.

Two bilateral P.A.G. records were made on 12 of each of four types of subject—normal males, normal females, abnormal males, abnormal females. As the above analysis showed that activity was minimum in the intermediate period, the neutral phase was redefined in these bilateral records as the intermediate silent period. All signals before and after the silent neutral phase were therefore included in the opening and closing phases. The maximum signal in the opening and closing phases of each channel was recorded. For each subject we thus had a total of 8 readings (from 2 phases by 2 sides by 2 records).

Elementary analysis indicated no significant differences at the 5 per cent level between the repeated readings from the two records for each type, phase and side combination. Readings from replicated traces were therefore combined, and differences be-

TABLE 1

ANALYSIS OF VARIANCE OF MAXIMUM SIGNAL HEIGHTS OF UNILATERAL P.A.G. RECORDS CONVERTED TO LOGARITHMS TO BASE *e*

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	VARIANCE RATIO			PROBABILITY *P*
				F	n_1	n_2	
Between types	1.501	1	1.501	< 1	1	18	$P>0.05$
Within types	60.083	18	3.338				
Between subjects	61.584	19	3.241	3.297	19	(180)	$P<0.01$
Within subjects	(176.957)	(180)	(0.983)				
Phases	50.174	4	12.544	12.359	4	72	$P<0.01$
Phase×type	3.982	4	0.996	< 1	4	72	$P>0.05$
Error (phase)	73.061	72	1.015				
Sides	1.650	1	1.650	3.293	1	90	$P>0.05$
Sides×phases	0.718	4	0.180	< 1	4	90	$P>0.05$
Sides×types	0.130	1	0.130	< 1	1	90	$P>0.05$
Sides×phase×type	2.145	4	0.536	1.070	4	90	$P>0.05$
Error (sides)	45.097	90	0.501				
Total	238.541	199					

TABLE 2

DIFFERENCES IN THE RANKED MEANS OF PHASES

	PHASE*					STANDARD ERROR
	N	C_1	O_2	O_1	C_2	
Mean logarithm	0.1352	0.9087	1.3009	1.3939	1.5150	0.1593
Equivalent reading (mm.)	1.145	2.481	3.673	4.030	4.549	1.173

* Means joined by a continuous line are *not* significantly different ($P \geq 0.05$).

tween sides for each phase and type combination were investigated. No significant differences were found except for normal females, where the mean of the right side (6.83 mm.) was significantly greater than the mean of the left side (4.88 mm.) in the closing phase. A test between affected and unaffected sides was possible in 18 abnormals, the remaining 6 being bilaterally affected. No difference between affected and unaffected sides was detected in either phase.

The orthodox skeletal and occlusal classification of each subject was recorded. Because of insufficient numbers, no statistical comparisons could be made between occlusal subdivisions of skeletal types. To estimate the effect of skeletal classification on unilateral records, the highly significant effect between subjects (Table 1) was divided into a component due to skeletal classes and a residual. The skeletal-class effect was significant at the 5 per cent level. Table 3 shows the mean logarithm in terms of the original readings (mm.) of the three skeletal classes. There was a definite trend in

TABLE 3

MEAN P.A.G. READING IN MM. FROM UNILATERAL AND BILATERAL RECORDS OF SUBJECTS WITH GIVEN SKELETAL CLASSIFICATION

Recording		Skeletal Class 1	Skeletal Class 2	Skeletal Class 3	Total No. of Subjects
Unilateral	Mean	2.12	3.38	4.22	
	No.	10	4	6	20
Bilateral	Mean	7.06	8.81	9.50	
	No.	28	10	10	48

mean P.A.G. reading by skeletal classification, skeletal class 1 having a mean significantly lower than that of skeletal class 3, as shown by the broken line in Table 3.

The 48 subjects on whom bilateral recordings were made were similarly grouped by skeletal classification. Their means showed a trend similar to that found in the 20 subjects recorded unilaterally. Direct comparison between the absolute sizes of the means from unilateral and bilateral records was not possible because of the different methods of measuring and summarizing unilateral and bilateral records. Although the skeletal means in bilateral records showed no significant differences by *t*-tests, when skeletal classes 2 and 3 were pooled the resultant mean (9.15 mm.) was significantly greater than the skeletal class 1 mean (7.06 mm.).

DISCUSSION

Ekensten[6] on an unselected series of 200 people found only 31 cases to be symptom-free. However, 89 patients were clinically diagnosed as abnormal and 134 by means of the phonogram. Thus crepitus is shown in his series to be the most common abnormal feature of joint function.

In the present investigation equal numbers of normal and abnormal cases, i.e., patients with pre-auricular pain, were examined, and no significant difference between

types was found. However, a repeatable individual pattern of noise was observed, but a normal joint, at the amplification used in this work, was not silent, as suggested by Ekensten.[6]

The sounds observed during opening were significantly greater than the sounds observed during the first phase of closing (Fig. 4). This finding may be explained by the passiveness of the lateral pterygoid muscle during the closing of the mouth. The sounds which occur during the final phase of closing cannot be considered in this analysis, as they are masked by the contacting noise of the teeth.

The increase in joint noise of skeletal class 3 subjects is interesting and may indicate some postural imbalance, with overaction of the lateral pterygoid muscle. There-

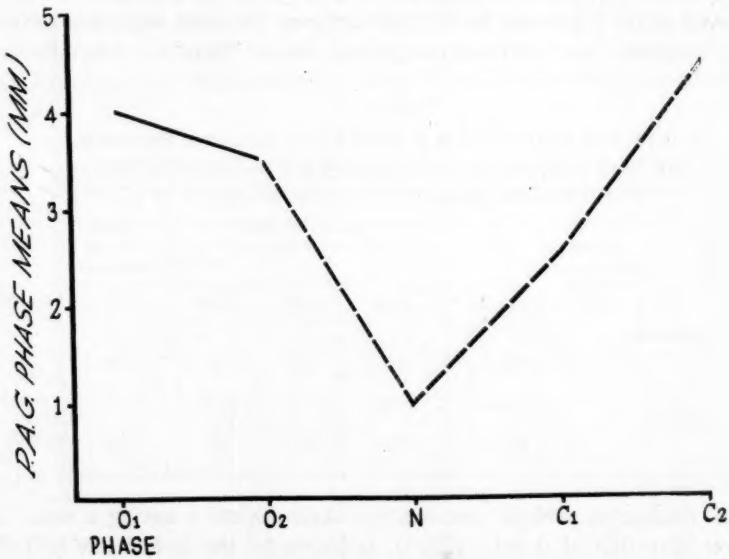

FIG. 4.—Phase means. *Solid line*—not significantly different; *broken line*—significantly different

fore, skeletal and possibly occlusal classifications are relevant to any study of joint noise and should be carefully standardized in a comparison of affected and unaffected subjects. This was not done in the present study, but it is unlikely, though not impossible, that the distribution of our subjects by skeletal classification (Table 3) could invalidate our investigation of the effect of abnormality in P.A.G. records.

The success of the phonoarthrogram as a record of joint sound has been proved, but the value of crepitus in the diagnosis of temporomandibular arthrosis and the clinical interpretation given to this sign by Ireland[1] must be reassessed.

SUMMARY

The phonoarthrogram (P.A.G.), a method of recording the sounds emitted by movement of the mandibular joint, is described, together with an apparatus to indicate the degree of mouth opening.

An analysis of the unilateral sound records of 10 normal and 10 abnormal sub-

jects who were known cases of temporomandibular arthrosis was made, irrespective of all other factors. No significant difference in the maximum sounds emitted between types was found.

The significant differences found between phases in this analysis were due mainly to the near-silence of the neutral phase. P.A.G. records were probably symmetrical, as comparable halves of the opening and closing phases were not significantly different. But halves of the closing phase were significantly different and the halves of the opening phase not significantly different, so there were some indications of asymmetry.

An analysis of the simultaneous bilateral sound records of 24 normal and 24 abnormal subjects with equal sex representation was made, irrespective of all other factors. This showed that no significant difference existed between repeated records on the same individuals or between sides, except in normal females during the closing phase. There were also no significant differences between affected and unaffected sides in abnormals.

An examination of individual P.A.G. means from both the unilateral and the bilateral records showed that there was a tendency for high readings to occur in skeletal class 3 subjects. The mean P.A.G. in skeletal class 1 subjects proved to be significantly lower than the mean P.A.G. in subjects allocated to skeletal class 2 or 3.

In general, this investigation shows that crepitus alone cannot be taken as a diagnostic sign of temporomandibular arthrosis.

Many thanks are due to the London Hospital Research Fund, which provided the equipment for this investigation, and to Mr. G. T. Hankey, under whose direction it was executed; also for assistance from the members of the London Hospital, the Royal Victoria Hospital, and the Queen's University clinical, teaching, and administrative staff.

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Millet (Cattail and Foxtail) as the Cariogenic Component of Experimental Rat Diets

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Interest in the cariogenic potential of cereal foods[1–3] and particularly the possible role of lysine in experimental caries etiology[2, 4, 5, 6] prompted these studies on the cariogenic effect of diets composed predominantly of millet. A common food, particularly in the Middle East, millet has been called "the poor man's cereal." Mangay, Pearson, and Darby[7] have demonstrated the striking lysine inadequacy of millet, and our current data agree with their finding. In the light of its predominant lysine deficiency, two varieties of millet were investigated for their cariogenic property. The effect of a dietary supplement of L-lysine and $CaHPO_4$ was also investigated.

EXPERIMENTAL METHODS

Following the general plan of our previous studies on experimental dental caries,[1, 2] the current experiments utilized Sprague-Dawley white rats weighing initially 30.0–35.0 gm. and at a weanling age of approximately 21 days. There were 35–40 littermates per each comparative group. The rats were housed 2 per cage and after 60 days on the experimental millet-base diets, fed ad libitum, they were sacrificed and dental caries diagnosed as previously described.[3] Since practically no caries developed on the upper molar teeth, caries experience is reported only for lower molars. The composition and analysis of the diets are presented in Table 1. In footnotes of this table there are data relative to the two kinds of millets used and their lysine content.

In addition to the studies on the cariogenic properties of millet, growth experiments were conducted to demonstrate the lysine deficiency of millet. In this study the basal diet contained 87.0 per cent millet "B," 2.0 per cent H.M.W. salts, 4.0 per cent cornstarch, 0.8 per cent glysine, 4.0 per cent cottonseed oil, and all the essential vitamins. A 1.0 per cent L-lysine supplement replaced 0.8 per cent glycine and 0.2 per cent cornstarch of the basal diet. The rats in this study were on the experiment 11 weeks. The growth data of this study, whose essential purpose was to demonstrate the lysine deficiency of millet as shown by rate of growth, are presented in Figure 1.

RESULTS

The major carious lesions observed in this study were located on lower buccal surfaces and were similar in appearance to what has been previously described.[3] The food intake of rats in experiments 3-c and 3-d averaged 6.4 and 5.6 gm. per rat daily, re-

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spectively. These low values provide evidence that the consumption of these diets was not sufficient for normal growth.

In experiments 1-a and 1-b (Table 1) practically all the rats developed caries, and both diets resulted in a similar number of carious teeth per rat. The highest severity score, i.e., 15.5, was obtained with the lysine-supplemented diet (1-b). A high incidence of caries also resulted in experiments 2-a and 2-b, but severity scores were lower than in experiments 1-a and 1-b. A different millet was used in 1-a and 1-b than in 2-a and 2-b, and a glycine supplement was added in 2-a, but it is not known that these differences would explain the difference in caries results. As in 1-b versus 1-a, the lysine supplement in diet 2-b versus 2-a again afforded no protection against caries.

The high incidence and severity of dental caries which developed with the 94 per

TABLE 1

COMPOSITION AND ANALYSIS OF MILLET DIETS—DENTAL CARIES RESULTS OBTAINED WITH AND WITHOUT LYSINE AND $CaHPO_4$ SUPPLEMENTS

	Experiment and Diet No.*							
	1-a	1-b	2-a	2-b	3-a	3-b	3-c	3-d
	Composition of Diets (Per Cent)							
Millet (A)†	80.0	78.0						
Millet (B)‡			78.0	78.0	94.0	94.0	76.0	76.0
Cerelose (commercial glucose)	18.0	18.0	18.0	18.0	0.0	0.0	18.0	18.0
Liver powder	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
L-Lysine–HCl	0.0	2.0	0.0	2.0	0.0	2.0	0.0	2.0
Glycine	0.0	0.0	2.0	0.0	2.0	0.0	2.0	0.0
$CaHPO_4$	0.0	0.0	0.0	0.0	2.0	2.0	2.0	2.0
	Analysis of Diets (Per Cent)							
Ash	1.37	1.30	1.87	1.87	3.89	3.89	3.57	3.57
Calcium	0.03	0.03	0.02	0.02	0.59	0.59	0.60	0.60
Phosphorus	0.26	0.28	0.28	0.28	0.72	0.72	0.66	0.66
Protein (N×6.25)	9.62	11.94	12.56	11.94	13.06	12.62	11.50	10.00
	Dental Caries Results							
No. of rats	38	40	38	37	37	34	36	37
Days on experiment	60	60	63	63	63	63	63	63
Average daily gain (gm.)	0.7	0.9	0.4	0.7	0.6	0.7	0.7	0.8
Rats having caries (per cent)	97.4	100.0	79.0	100.0	78.3	88.2	73.0	75.7
Carious teeth per rat	3.9	4.4	2.2	3.7	2.6	3.2	2.3	2.6
Standard error	± 0.31	± 0.31	± 0.27	± 0.23	± 0.31	± 0.31	± 0.33	± 0.34
Caries severity score per rat	11.6	15.5	3.6	7.7	6.9	13.1	3.3	5.6
Standard error	± 1.58	± 1.96	± 0.51	± 0.75	± 1.35	± 2.16	± 0.56	± 0.98

* Vitamins A, D, and E were given orally once a week.

† Cattail millet (*Pennisetum glaucum L.*), grown in Montgomery County, Maryland. Contains 1.93 per cent nitrogen, 2.32 mg. lysine per 16 gm. nitrogen.

‡ Foxtail millet (*Setoria italica*), obtained from Tennessee Milling Company and grown in Kansas. Contains 1.81 per cent nitrogen, 2.28 mg. lysine per 16 gm. nitrogen.

cent millet diets (3-a and 3-b) is of special interest, since these diets contained no cerelose (commercial glucose). Since the animals in 3-a, 3-b, 3-c, and 3-d were littermates, the cariogenicity of the non-cerelose diets (3-a and 3-b) becomes more impressive by direct comparison of diets 3-a and 3-b versus 3-c and 3-d. These four diets were also made adequate in calcium (0.6 per cent) and phosphorus (0.7 per cent) by a supplement of 2.0 per cent $CaHPO_4$. Diet 3-b containing 2.0 L-lysine resulted in a greater caries severity score than did diet 3-a, which was lysine-deficient. Further evidence of a failure of L-lysine–HCl, as well as $CaHPO_4$, to inhibit the dental caries as developed by these particular millet-base diets is evident in experiments 3-c and 3-d.

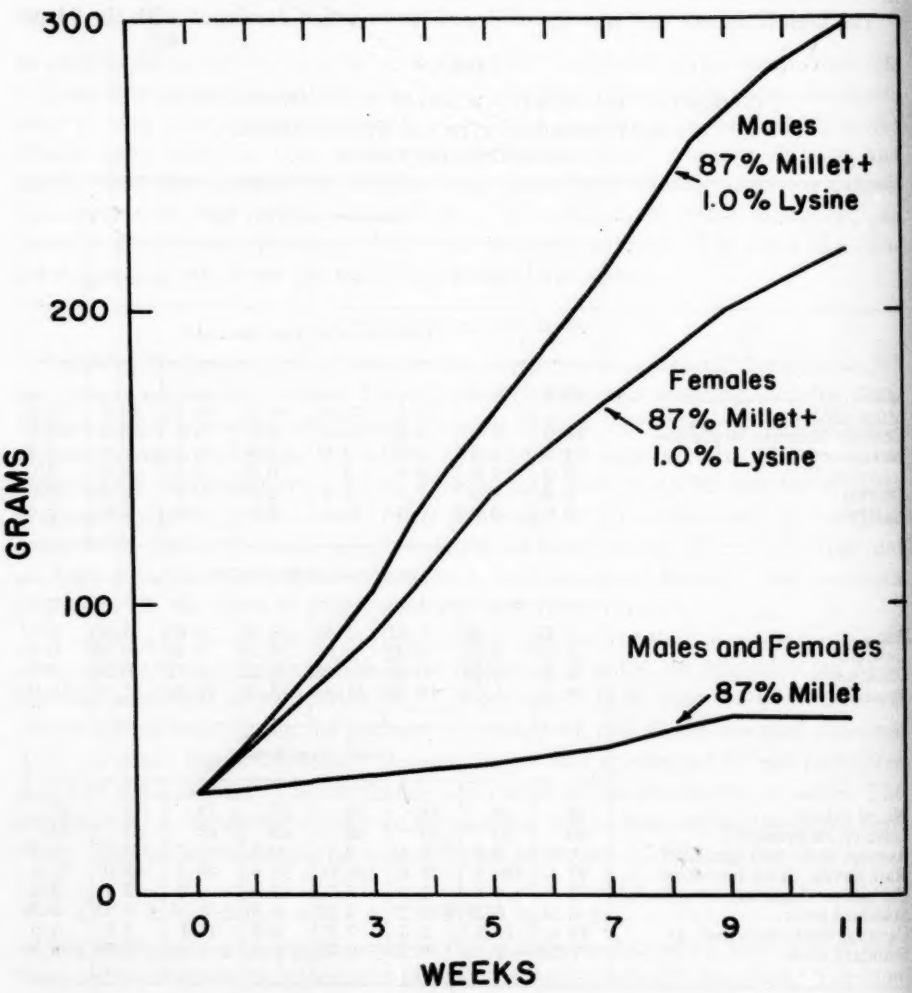

FIG. 1.—The effect of lysine supplement on rate of growth of rats fed an adequate diet containing 87 per cent millet "B."

DISCUSSION

In the light of other experimental caries studies from this laboratory, the results with these millet-base diets were somewhat unexpected. Previously, in a considerable number of trials, caries was significantly reduced by the addition of lysine to lysine-deficient skim-milk powder diets,[4] to cereal diets,[2, 4, 5] and to a lysine-deficient, zein-base diet.[6] Growth failure as well as cariogenicity, was corrected by lysine supplementation of these diets. Prior to obtaining the data shown in Table 1, the lysine inadequacy of our millet "B" was demonstrated. The growth data are shown in Figure 1. This result agrees with the data of Magnay, Pearson, and Darby,[7] who used a millet of the same source as our millet "B." On neither of these diets, whose composition is stated above, was there any development of dental caries. In the light of our previous results,[8] perhaps two factors present in this nutritionally adequate diet, i.e., the 4.0 per cent cottonseed oil or the H.M.W. salt mix, may have prevented the development of dental caries. Also these rats were housed individually, which is not so conducive to caries development as is multiple housing.

With regard to the status of L-lysine as a cariostatic agent, it is not known whether an uncomplicated lysine deficiency or perhaps some other factor associated with the heat processing of foods is being corrected whenever an L-lysine supplement reduces dental caries. Thus far our success in inhibiting experimental caries by an L-lysine supplement has been most pronounced and convincing when either a heat-processed-cereal food or a heat-processed skim-milk powder was the main component of a basal cariogenic diet. The millet of these diets was not heat-processed. While it seems likely that a lysine deficiency was not responsible for the caries which developed on these obviously inadequate millet diets, this cannot be an unequivocal conclusion. Unresolved at this time are the factors of this particular inadequate millet-base diet which caused the dental caries and failed to respond to a 2.0 per cent lysine supplement. The presence of cerelose evidently was not responsible for the cariogenicity of this particular millet diet. Perhaps the low protein content was a factor.[6]

In addition to the observations regarding a lysine supplement, the noncariostatic effect of $CaHPO_4$ also has considerable interest. Diets 3-a, 3-b, 3-c, and 3-d, as previously mentioned, contained 0.60 per cent calcium and 0.70 per cent phosphorus, but nonetheless they remained significantly cariogenic. This result—pertinent to this particular diet—agrees with our previous data indicating that $CaHPO_4$ is not cariostatic under all dietary conditions.[2, 3, 9] As in our other diets,[2, 9] $CaHPO_4$ might have become effective, had NaCl been added to the diet.

These experiments obviously call to mind the ever disturbing possibility that a diet may have little or no effect on the development or prevention of caries by reason of a systemic association but may act directly within the oral cavity. Diet components may react on oral tooth surfaces, of which the destructive action of acid drinks and beverages is an extreme example;[10] they may possibly alter the properties of the saliva; or they may influence particularly the complex nutrition and metabolism of the oral flora. The possibility of such a local oral reaction needs particular thought when experimental cariogenic diets include items such as freely available mineral salts, amino acids, oils, fats, and carbohydrates.

The number of different types of experimental diets having pronounced cariogenic properties is ever increasing. The mechanism by which these various diets can produce caries may encompass a number of variable processes. Their very diversity may well account for a great variability in response to "anticaries" agents.

SUMMARY

An inadequate diet composed essentially of a cereal food, i.e., millet, with and without the presence of 18 per cent cerelose (commercial glucose) in the diet, was fed to white rats for approximately 60 days. Seventy-five to 100 per cent of the rats developed relatively severe dental caries, most generally on the buccal surfaces of lower molar teeth. The cause of this cariogenicity was not ascertained.

The pronounced lysine inadequacy of millet was demonstrated. Nonetheless, a supplement of 2 per cent lysine did not inhibit the dental caries as produced by this millet-base diet which apparently was inadequate in other respects besides lysine.

Although not based on a control versus test-group comparison, 2.0 per cent $CaHPO_4$ apparently had no preventive effect on the cariogenicity of this millet diet.

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Prenatal Growth and Morphology of the Human Bony Palate

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The present study was directed toward gaining an understanding of the course of normal development of the human bony palate. It was undertaken as part of a program investigating the developmental processes involved in cleft palate. The philosophy underlying this approach holds that only by delineation of what is normal in development can abnormalities be detected and more fully investigated. Discussing cleft palates, Patten[1] (p. 735) states: "By comparing these defects with the normal course of development of the region involved, it is quite obvious that the defect is located at a point where in the normal course of events a fusion should have occurred, and that the defect itself is a result of the failure of this fusion to go to normal completion." In the introduction to their study of the embryology of cleft palate in mice, Walker and Fraser[2] emphasize the point that "to understand the abnormal it is usually necessary first to understand the normal."

The normal developmental processes of closure of the secondary palate have been described recently by Walker and Fraser[3] in mice of strains A/Jax, C57BL/Fr, and DBA/1. The classical concept of secondary palate development in man was promulgated in the latter half of the nineteenth century by His[4] and Dursey.[5] With the exception of the work by Walker and Fraser,[2] none of the theories of secondary palate development has been based upon large numbers of fetuses; hence there has been no statistical demonstration of norms.

Studies of the development of the bony palate have concentrated primarily upon the number of centers of ossification found in the premaxilla, the maxilla, and the palatine bone. Until Woo's paper[6] on "Ossification and Growth of the Human Maxilla, Premaxilla and Palate Bone" there had been no work on the developmental changes and relationships of the three bones of the palate based upon adequate samples and utilizing the clearing (KOH) and staining (alizarin red S) technique.

Woo's avowed object in his 1949 paper was to describe "the process of ossification, the formation and eventual obliteration of the facial premaxillary bones, the growth of the premaxilla, maxilla and palate bone and their relationship." It was obviously impossible, in a short paper, to describe the palatal processes of these bones from the beginnings of ossification to final formation of the bony palate, nor did Woo concern himself with metric analysis.

Woo's conclusions regarding the formation of the hard palate may be summarized as follows:

1. The palatine process of the premaxilla, including the infravomerine process, extends posteriorward almost to the vomer bone. It forms the anterior portion of boundaries of the incisive foramina and the elevated part of the nasal crest.

It lies under and supports the anterior, pointed extremity of the vomer. The infravomerine process united with the medial part of the palatine process forms the anterior wall of the incisive foramen.

2. At the end of the seventh week the bony lamella radiates posteromedially to form the palatine process and downward and inward to form the internal alveolar wall.

During the ninth week a bony lamella radiates medioposteriorly from the maxillary center above the canine tooth, the horizontal portion of which unites medially with the palatine process but remains separate from it laterally.

At the end of the third month the palatine portion has greatly thickened.

3. From a tiny crescent-shaped ossification center, the palatine bone extends rapidly mediad ward to form the horizontal plate, about the end of the seventh week. At the tenth week at the junction of the vertical and horizontal plates the bone extends forward and begins to overlap the maxillary bone. The horizontal plate is of a quadrilateral form, the medial side of which is about three-fifths of the lateral side.

Woo remarks that the "anterior portion of the palate bone which overlaps the maxillary bone has been neglected in textbooks of human anatomy."

In another paper,[7] Woo points out that "the palatine processes of the maxillae and the horizontal parts of the palate bones overlap in the extent of their bevelled edges with the maxillary portions in the inferior position. Thus, the articulation belongs to the type of *squamous* suture instead of *serrate suture*."

Woo's observations are, in general, fully substantiated by the investigations of the present author. The major purpose of this paper is to outline in more detailed form the growth and morphology of the bony palate in its developmental sequence and to describe its differential growth characteristics in quantitative terms. The significance of these developmental norms as a background for the study of the osseous morphology of cleft palates will be discussed.

In the term "bony palate" we include the alveolar arch of the maxilla, as well as the palatal processes of the maxilla, premaxillary, and palatine bones.

MATERIALS AND METHODS

A series of 151 embryos and fetuses ranging in age from 7 to 18 weeks was cleared and stained simultaneously with KOH and alizarin red S according to the method of Noback and Noback.[8] Age was estimated from crown-rump length.[9] The stained palate of each specimen was photographed in color from the inferior aspect, with the palatal plane oriented parallel to the film. Transmitted light was used to achieve maximum contrast. A clear plastic scale (in millimeter units) was included in each photograph in the plane of the palate. This permitted direct measurements from the photograph.

Studies of the morphology of the palate were based on direct microscopic examination of the specimen, together with palpation. Colored slides were projected, to facilitate over-all examination and comparison of different specimens. The palates were classified according to developmental stages arbitrarily set up by certain marked morphologic plateaus attained by one or more of the three bony processes involved.

A number of anatomical landmarks were chosen and described. Diameters between certain of these landmarks were measured on all the palates. Statistical analysis of various combinations of these diameters was performed, in order to describe norms of interrelationships between different components of the growing bony palate.

RESULTS AND DISCUSSION

Morphology of the growing bony palate.—Woo[6] found in a series of 84 human embryos and fetuses one specimen showing a separate center of ossification for each premaxilla. In our series of 151 specimens, two such examples of approximately 7 weeks of age were noted (Fig. 1). In an earlier paper[10] the present investigator described the beginnings of osseous maxillary development as follows:

> Both the premaxillary and maxillary centers of ossification appear in what eventually might be termed the maxillary corpus,—that portion of the maxilla from which both the frontal and alveolar processes will arise. Once the two centers on either side have fused to form the corpus there is no longer any morphologically separate premaxilla. However, processes which arise from the mesial and distal portions of the corpus may be identified as premaxillary or maxillary without connoting developmental origin. Anatomically there is only a single osseous entity by the end of the 8th week.

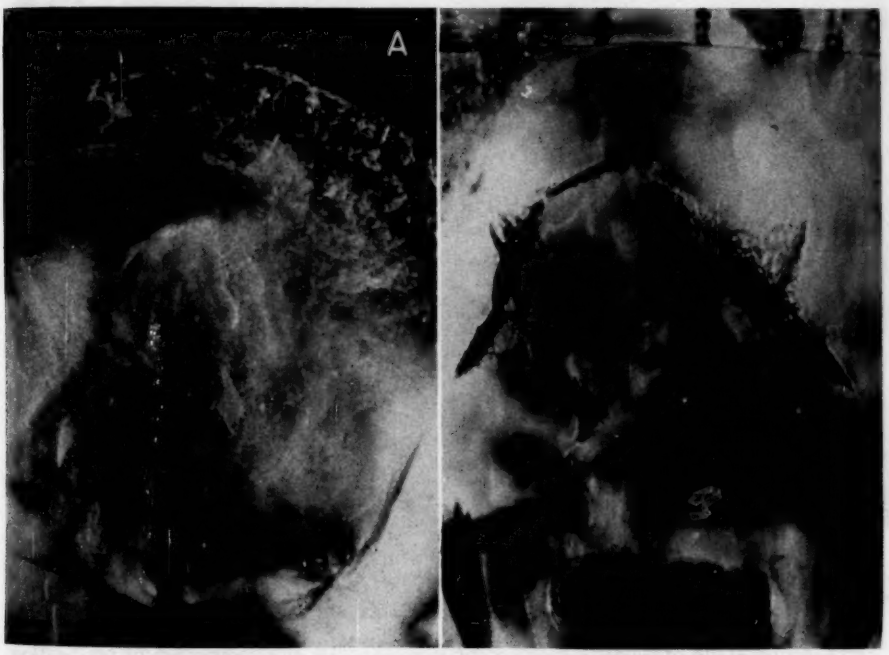

FIG. 1.—Seven-week-old human embryos. *A*, palatal view, showing separate premaxillary bones; *B*, palatal view, showing premaxillary bones almost at moment of fusion with maxillae.

From the maxillary corpus, by the ninth week, there radiates medially a network of bony trabeculae. This network arises in the area of the future canine alveolus and is characterized by a longer, more heavily stained vertical plate, which is directed posteriorly from a point on the maxillary corpus marking the future distal canine alveolus. From the same point another shorter, less heavily stained vertical plate proceeds in an anteromedial direction. From an inferior aspect, the site of origin of the two bony lamellae presents a K-shaped configuration, the vertical back of the K being represented by the maxillary corpus. The inferior margins of the two arms of the K will form the

future lingual alveolar crests. The distal canine alveolus, which is marked thus early by the intersection of the two arms of the K, will henceforth be referred to as the "K point." The earliest manifestation of the K point, accompanied by the beginnings of the radiating network of bony trabeculae, marks the first stage in the development of the bony palate. At this time, both the premaxillary and the maxillary frontal processes are clearly apparent but ununited, the infravomerine processes have not yet appeared, and the palatine bones appear as crescent-shaped, anteroposteriorly oriented bony palates. The internal pterygoid plates appear as small, round centers posterior to the palatine bones (Fig. 2).

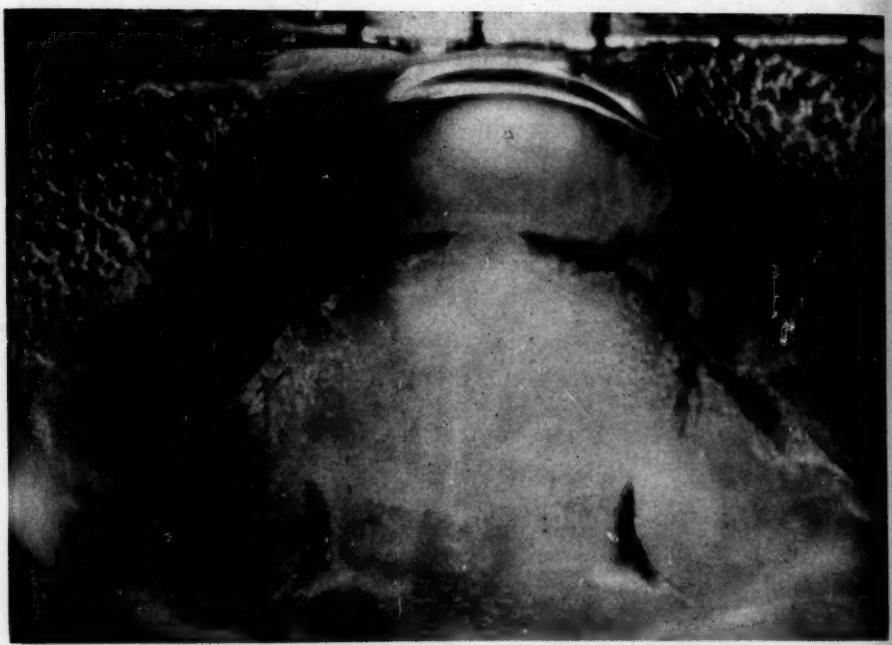

FIG. 2.—Ten-week-old human fetus, showing the first stage of development of the bony palate. Premaxilla and maxilla are fused and have sent up frontal processes. The K point is visible, and the palatine bones appear as crescent-shaped plates.

The second stage of palate development is marked by the beginnings of ossification of the alveolar crypts of the central and lateral incisors. The process originates at the premaxillary portion of the corpus and appears as two open rectangular zones separated from each other at the mid-line. They mark the future apical portions of the alveolar sockets. The premaxillary and maxillary frontal processes are still clearly separated, and a thin trabecularized network of bone has developed superior to the premaxillary portion of the corpus to form the subnasal external maxillary wall. The palatal processes of the maxillae have continued to radiate mesially, anteriorly, and posteriorly but are still separated from each other by a large hiatus. In this hiatus and on either side of the mid-line, the two spindle-shaped vomer bones have appeared. The area of the canine alveolus is still completely unossified. Bony trabeculae have radiated

anteromedially from the palatine bones to form the horizontal palatal processes of those bones. They have not yet met in the mid-line and have contacted, but not yet overlapped, the adjoining palatal processes of the maxillae. From the inferior aspect the palatine bone presents a triangular appearance, with the base formed by the heavily stained vertical plate of the bone and the apex directed toward the mid-line. The internal pterygoid plates present a crescent-shaped appearance at this time and are not in contact with the palatine bone (Fig. 3).

FIG. 3.—Nine-week-old human fetus, illustrating the second stage. The horizontal plates of the palatine bones and maxillae are spreading medialward, the vomer bones are present but ununited, and the alveolar crypts of the upper central incisors are forming.

The third stage is characterized by the appearance of the infravomerine processes, which, according to Woo,[6] arise from separate centers and fuse quickly thereafter with the medial portion of the palatine process of the premaxilla. In the present series the origin of these processes as independent ossification centers was not noted. Palpation of the infravomerine processes at this stage indicates that they are attachd to the developing alveolus of the central incisor at the mesiolingual corner of the rectangular zone which appeared in stage 2. The vomer bones are now fused in their posterior half, so that the two wings are united. The K points are well demarcated, the upper arms

curving lateralward to form the lingual alveolar margin of the canine. The lower arms extend posteriorly, lying lateral to and paralleling the anterior half of the vertical plate of the palatine bone. From the mid-point of the upper arm of the K another heavily stained bony trabecula extends medially, to form what eventually will be the anterior border of the palatal process of the maxilla. Between this bony extension and the lower arm of the K there is a more lightly stained network of bone fanning out medially and posteriorly. This network is the developing palatal process of the maxilla. The lower arm of the K has proceeded distally to form the lingual aveolus of the first primary molar. Between the buccal and lingual alveolus of the first primary molar can be seen a darkly stained area which represents the facial surface of the maxilla. As yet, there is no septum formed between the central and lateral incisors. The K point, however, marks the buildup of the septum between the canine and the first primary molar. The palatine bone is now developed in more detail. The posterior border of the horizontal plate is heavily stained and continues laterally to form the well-delineated pyramidal process. The vertical plate, particularly the spenoidal process, is observed posterior to the horizontal process. The latter has continued to spread medially toward the midline but has not yet proceeded as far as the wing of the vomer. Anteriorly, it has now overlapped slightly the palatal process of the maxillary bone (Fig. 4).

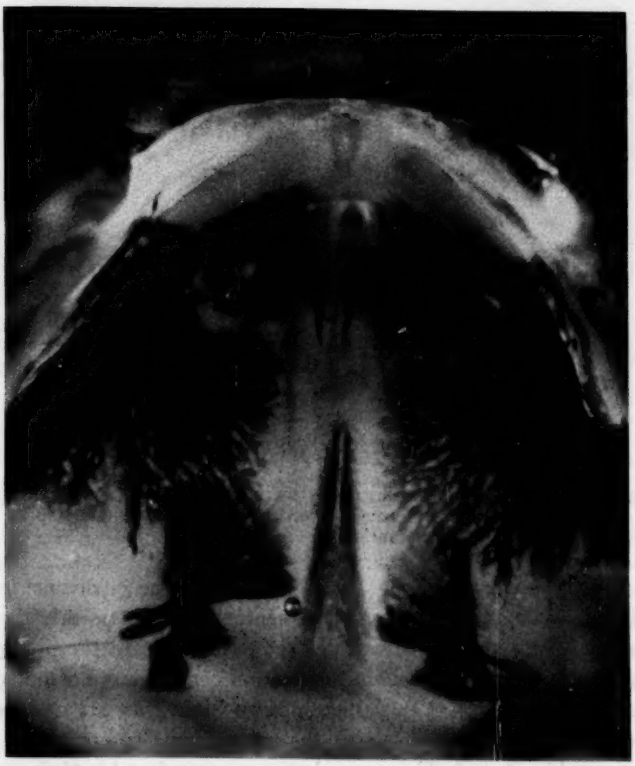

FIG. 4.—Ten-week-old human fetus in the third stage of palate development. The horizontal plates of the palatine bones are beginning to overlap those of the maxillae. The alar portions of the vomer bones have fused, the infravomerine processes are ossified, and the lingual margins of the alveoli of the first primary molar are formed.

Stage 4 is marked by a number of features that appear concurrently. The alveolar sockets are increasing in depth, that is, growth of the walls is proceeding inferiorward. This is indicated by the darker lingual and distal alveolar borders of the incisors and the lingual alveolus of the primary first molar. In addition, the buccal wall of the first primary molar is curving lingually, to form the distal septum. The buccal and lingual alveolar borders of the cuspid are not yet defined, nor has the septum between the central and lateral incisors begun to ossify. However, there is now apparent a pointed bony process midway on the lingual alveolar border of the central and lateral incisors directed medially. This process will eventually form the anterior bony margin of the incisive suture. The horizontal process of the palatine bone has now extended as far as the wing of the vomer and has overlapped the palatal process of the maxilla for a distance corresponding to the length of the posterior half of the socket for the first primary molar. The palatal process of the maxilla has not proceeded quite as far medially as has the analogous process of the palatine bone. The pyramidal process and the vertical plate of the palatine bone appear as heavily stained, long, laterally directed spicules. The pterygoid hamulus is now distinguishable (Fig. 5).

In stage 5 the left and right palatal processes of the maxilla and palatine bones have reached almost to the mid-line and have achieved their maximum overlap. The area of overlap is clearly distinguishable. The horizontal process of the palatine bone overlaps the palatal process of the maxilla in such a way that the two thicknesses of bone show as a lighter area and present a butterfly-shaped appearance. The narrowest stretch of overlap is at the mid-line. The greatest length of overlap is along the lateral edges, extending from point K posteriorly to the area just anterior to the base of the pyramidal process. In stage 5 the palatal surface, as seen from below, is divided into three zones. The anterior zone consists of the deeply stained trabeculae of the maxilla, which radiate from the lingual alveolus of the canine toward the mid-line; the middle zone described above, which consists of a superior layer of palatine bone and an inferior layer of maxillary bone and is lightly stained; and the posterior zone, which is formed by the horizontal trabeculae of the palatine bone as they radiated from the region of the pyramidal base toward the mid-line. At this time the space between the two halves of the anterior zone is slightly wider than that between the halves of the other two zones.

The buccal alveolar borders of the incisors, canine, and primary first molar have now assumed their characteristically curved shape, and the buildup of the lingual alveolus is noticeably advanced over the previous stage, particularly for the incisor region. The lateral half of the incisive suture is more sharply defined. The vomer bone can no longer be distinguished as such, but its presence causes a deeper stain along the medial edges of the palatal processes. The beginning formation of the septum between the central and lateral incisors can be seen as slight projections from the labial and lingual alveolar margins. The lingual and distal aveolus of the canine has not yet taken shape, nor has the distal alveolus of the primary first molar yet appeared. The posterior border of the bony palate is straight and at right angles to the sagittal plane. The medial edge is rounded, so that there is no suggestion at this stage of a posterior nasal spine. The pterygoid process has enlarged and is closer to the palatine bone. The beginnings of the fossa can be distinguished (Fig. 6).

In stage 6 the two halves of the palatal processes are in apposition at the mid-line, although no fusion has taken place and the two processes are generally not in contact.

The three zones effected by the overlapping of the maxilla by the palatine bone are still clearly visible. The septa of the alveoli from the mesial alveolus of the central incisor to the distal of the primary first molar have begun to ossify from both buccal and lingual aspects. The lingual alveolus of the second primary molar has almost completed its ossification, but the buccal alveolus is just in the beginning stages of development. The posterior border of the palatal process of the palatine bone now shows its characteristic anterior convexities, so that the site of the posterior nasal spine is a rounded projection in the mid-line. At this stage the calcified tips of the mesiobuccal cusps of the first primary molars are visible, together with the calcified portions of the crowns of the central incisors. Normally, at this stage, the lateral incisor, canine, and second molar have not begun calcification.[11] The border of the future incisive foramen has now begun to assume its characteristic shape, although it is relatively larger than

FIG. 5.—Eleven-week-old human fetus, representing the fourth stage of development. The buccal and lingual walls of the alveolar crypts of the incisors and the first molar are formed, but the septa are not yet ossified. The horizontal plates have extended more medialward and are beginning to obscure the vomer bones. The pyramidal processes of the palatine bones are readily apparent.

it will eventually be. The infravomerine processes now appear only faintly, since they are located superior to the bony structures of the palate and anterior alveoli (Fig. 7).

Among the 17- and 18-week-old specimens the succeeding stage 7 is represented primarily by more sharply defined borders of the incisive suture and foramen, the buccal and lingual alveoli, and the alveolar septa. Both buccal and lingual aveoli are more heavily trabecularized, hence more deeply stained. The buccal alveolar rim of the second primary molar is roughly only about one-quarter formed. The right and left palatal processes of the maxillae and palatine bones have not yet met in the mid-line. The other features remain very much the same as depicted in stage 6 (Fig. 8).

The above seven morphological stages in the development of the bony palate can be described in the period between 9 and 18 weeks of prenatal life. The assignment of

FIG. 6.—Thirteen-week-old human fetus in the fifth stage of palatal development. The horizontal processes of the palatine bones have achieved their maximum overlap of the maxillary horizontal processes, and both processes almost meet along their entire length in the mid-line. A light "butterfly" pattern marks the area of overlap of the two horizontal plates. The buccal margins of the alveoli of the incisors and cuspid have assumed their characteristic curvilinear outline.

a specimen to any particular stage should be done on the basis of the concomitance of traits rather than by placing reliance upon any one trait. There was very little correlation of stages to age in weeks as estimated by crown-rump length. Apparently, palatal development may be considerably precocious or retarded with reference to chronological age within the 9-week period studied. On the other hand, there was no difficulty in placing the 151 specimens in the developmental categories, since the traits described for each stage are remarkable cohesive.

Once the presumably "normal" morphologic configurations through which the developing bony palate passes have been delineated, it becomes comparatively simple to recognize deviations from the pattern and to assign such a deviant specimen to one of

FIG. 7.—Fourteen-week-old human fetus in the sixth stage. The lingual alveolus of the cuspid has now formed, and the septa between the first four primary teeth have begun to ossify. The posterior border of the bony palate is well demarked. Only the buccal alveolar margin of the first molar is yet unossified. The calcified tips of the mesiolingual cusps of the first molar are visible.

the seven stages. One example is offered to illustrate the possible use of these "norms." A fetus was observed with a postalveolar cleft. When this specimen was cleared and stained, it presented the appearance noted in Figure 9. The criteria useful for assignment to the proper developmental stage are confined to the alveolar structures and to the vomer and sphenoid bones. The maturational status of the alveolar septa, the size and configuration of the pterygoid process of the sphenoid, and the development of the wings of the vomer bone suggest that this specimen was well along in stage 6. The fetus, however, is apparently only 13 weeks of age, judging from its crown-rump length of 87 mm. It is a female. There are several interesting features presented by this specimen. Some of these are directly observable in the figure; others can be detected only by palpation under a microscope. On both sides the horizontal process of the palatine

bone has overlapped that of the maxilla to a more anterior extent than is the case in non-cleft specimens. The most anterior point on the palatine bone is located at the lingual alveolus of the canine. The medial border of each side of the bony palate appears as if cut with a knife, the stroke running from the posterior margin of the incisive foramen lateroposteriorly to the posterior border of the horizontal process of the palatine bone. The fan-shaped appearance of the trabeculae which radiate from the K point (in the case of the maxilla) and from the base of the pyramidal process (in the case of the palatine bone) is preserved. However, there is a gap between the anterior third and posterior two-thirds of the horizontal process of the maxilla. On the right side this gap

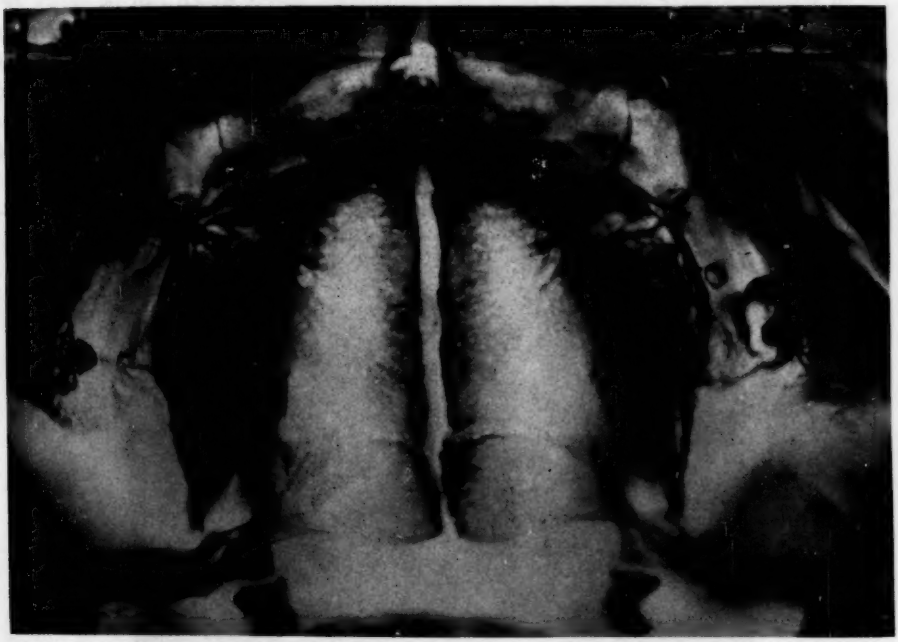

FIG. 8.—Fifteen-week-old human fetus in the seventh stage. There is better definition of the anatomical structures already apparent in stage 6. The buccal alveolar margin of the first molar has begun to ossify.

permits one to lift the anterior third without disturbance to the posterior two-thirds. On the left side, bony fusion between the two segments has occurred, so that functionally the entire horizontal process is a single plate of bone. The incisive suture cannot be seen in the photograph. This is because the entire lingual alveolus of the lateral incisor overlaps the suture from below. This is a bilateral phenomenon which has not been observed in other specimens. We can only note that the lingual alveoli of the two lateral incisors seem to be detached from the surrounding alveolar structures in such a way that the lingual aspect is thrown into an inferior overlapping position with respect to the area of the incisive suture.

A final observation needs to be added. It was noted that in stage 3 the palatal process of the palatine bone begins to overlap that of the maxilla. Soon after this over-

lapping takes place, a bony ridge begins to form on the inferior side of the palatal process of the palatine bone. It builds up along a transverse line demarking the most posterior extent of the palatal process of the maxilla. On the superior aspect of the maxilla a similar ridge begins to build up along a transverse line demarking the most anterior extent of the palatine process of the palatine bone. These transverse ridges are presumably primary sites of postnatal fusion between the horizontal plates of the maxillae and palatine bones. It is the most posterior ridge that marks the site of the transverse palatine suture as observed in the postnatal skull. Only by palpation of the stained specimen can the two ridges be observed.

In summary, there appear to be seven morphologic levels which can be easily described as the palate and associated alveolar structures undergo ossification from their various centers between the prenatal ages of 9 and 18 weeks. The maturational status of the morphologic criteria for each stage is quite consistent, although there is no strong correlation of these stages with age as estimated from crown-rump length. Having set forth the sequence of morphologic patterns during the osseous development of the palate, we next turn our attention to the question of growth rates.

Quantitative aspects of growth of the bony palate.—The conventional anthropo-

FIG. 9.—Thirteen-week-old human fetus with a cleft bony palate. The criteria of bony palate development indicate that the specimen is in the sixth stage.

metric landmarks used in measuring the postnatal bony palate[12] cannot be located in the developing prenatal bony palate; hence new landmarks and sets of measurements had to be devised. Eight dimensions (Fig. 10) were pragmatically established, keeping in mind the stricture that they are not necessarily vectors of individual bone growth, nor are they themselves true anatomical entities.

Measurements were taken on all the specimens of the sample wherever the landmarks involved could be definitely located. Product-moment correlations between these measurements and crown-rump length were not calculated, inasmuch as equal crown-rump intervals are not necessarily representative of equal intervals of time. Rather it

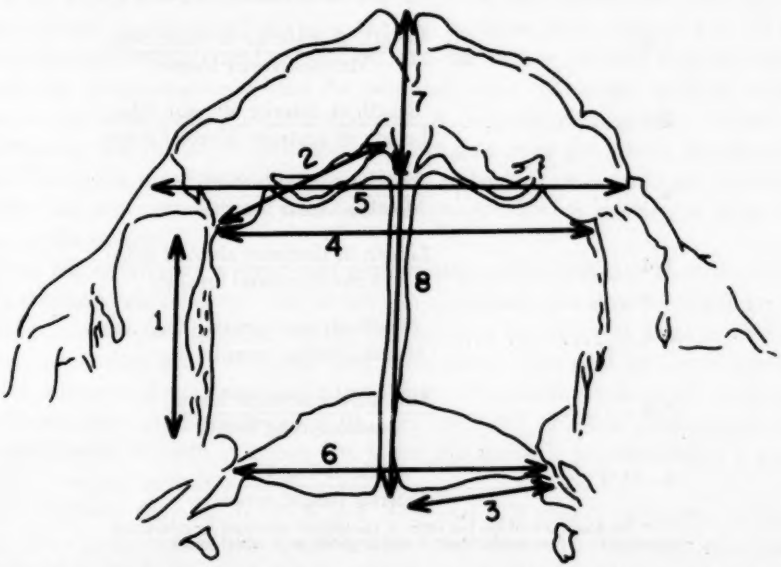

FIG. 10.—Drawing showing eight dimensions pragmatically established to measure growth of the bony palate. *1* = length of posterior alveolar ridge; *2* = length of anterior alveolar ridge; *3* = breadth of posterior border of palate; *4* = anterior palate breadth; *5* = maxilloalveolar breadth; *6* = posterior palatal breadth; *7* = mid-sagittal alveolar length; and *8* = maxilloalveolar length.

was felt that plotting the ratios between each pair of measurements against one other dimension would present a picture of the changing interrelationships of palate dimensions against an arbitrarily selected diameter of the palate itself. A curved line resulting from the co-ordinates of, say, maxilloalveolar length and crown-rump length is not necessarily indicative of differential growth rates. Therefore, attempts to determine growth rates in terms of absolute increments of time were abandoned. Instead, by means of plotting indexes against a selected palatal dimension, the differential growth rates taking place in the palate as the palate itself increased in size, irrespective of absolute age, were described.

Maxilloalveolar breadth was selected as the diameter of reference throughout, and it appears as the abscissa on all graphs. The indexes used are listed and described in Table 1. The resultant data were analyzed for significance of linear regression, utilizing the *t*-test (see Table 2).

TABLE 1

LIST AND DESCRIPTION OF INDEXES

No. of Measurement	Description of Measurement
7/2	Mid-sagittal alveolar length / Length of anterior alveolar ridge
6/4	Posterior palatal breadth / Anterior palatal breadth
2/4	Length of anterior alveolar ridge / Anterior palatal breadth
1/8	Length of posterior alveolar ridge / Maxilloalveolar length
2/1	Length of anterior alveolar ridge / Length of posterior alveolar ridge
4/5	Anterior palatal breadth / Maxilloalveolar breadth
1/4	Length of posterior alveolar ridge / Anterior palatal breadth
8/5	Maxilloalveolar length / Maxilloalveolar breadth
7/8	Mid-sagittal alveolar length / Maxilloalveolar length
8−7*/4	Palatal length / Anterior palatal bread

* The numerator of the last index in the table is calculated by subtracting measurement 7 from measurement 8, giving mid-sagittal palatal length.

TABLE 2

SIGNIFICANCE OF LINEAR REGRESSIONS OF PALATAL INDEXES ON MAXILLOALVEOLAR BREADTH

Palatal Index	No. of Specimens	*T*-Value	*P*-Value
7/2	95	0.76	0.30–0.50
6/4	85	0.234	0.50–0.70
2/4	103	6.48	0.001*
1/8	90	3.66	0.001*
2/1	101	4.00	0.001*
4/5	100	12.69	0.001*
1/4	101	11.56	0.001*
8/5	94	1.87	0.05–0.10
7/8	92	1.00	0.30–0.40
8−7/4	93	5.28	0.001*

* The slope differs significantly from zero at the 0.1 per cent level.

The interpretation of Table 2 may best be explained by using index 7/2 as an example. This index expresses the relationship of mid-sagittal alveolar length to the length of the anterior alveolar ridge. For any one fetus the co-ordinate is the product-moment of this value and the value of maxilloalveolar breadth (measurement 5). Plotting of the 95 co-ordinates shows a scattering of points which appear to cluster about a horizontal axis. Analysis of linear regression shows a least-squares line with a negative slope of 0.003. This would appear to indicate that, as the maxilloalveolar breadth increases, the ratio of mid-sagittal alveolar length to the length of the anterior alveolar ridge decreases at the rate of 0.3 per cent to every millimeter. However, as shown in Table 2, the slope cannot be shown to be different from zero. Apparently, then, the ratio remains constant as the maxilloalveolar breadth increases from roughly 3 to 19 mm.

Any horizontal least-squares line indicates that the ratio in question does not change. This does not mean, obviously, that the two dimensions that make up each ratio are growing at the same rate; quite the contrary. A constant ratio of, say, 50 per cent would indicate that the larger dimension is growing at twice the rate of the smaller as the maxilloalveolar breadth increases. Similarly, a constant ratio of 33 per cent would mean that the larger dimension (placed in the denominator) is growing at three times the rate of the smaller.

A linear regression with a significant positive slope would indicate an index increasing at a constant rate relative to the increase in maxilloalveolar breadth. An index with increasing values means that the smaller dimension is increasing at a faster rate than the larger dimension, hence is approaching equal value. That is, if the trend continued, the index value would equal and then exceed unity. A negative slope, on the other hand, would show a constant decrease in the value of the index, and this would signify that, as maxilloalveolar breadth increases, the larger dimension is accelerating at a greater rate than is the smaller one.

Table 2 reveals that indexes 7/2, 6/4, 8/5, and 7/8 cannot be shown to regress linearly upon measurement No. 5 with a slope other than zero. However, examination of the distribution of co-ordinates on the graph suggests that a curvilinear, rather than a linear, regression might better fit the data in the cases of 7/2 and 6/4. Accordingly, in each case, the means of the indicial values that occurred at each 2-mm. interval of maxilloalveolar breadth were plotted and connected by straight lines. The results for 7/2 and 6/4 are shown in Figure 11. In the former, there is a sharp decline and then a leveling-off (Fig. 11, *A*). In the latter there are two peaks separated by a long shallow valley (Fig. 11, *B*). Before we speculate about the meaning of these curves, it must first be determined whether or not the data show significant curvilinear regression.

Analysis of variance for curvilinear regression was performed on the following indexes: 7/2, 6/4, 2/4, and 1/8, since the plotting of means for these indexes suggested the possibility of curvilinear regression (Fig. 11). Table 3 presents the results of the analyses of variance on each of the four indexes. It can be seen that curvilinear regression in each case is significant at the 0.1 per cent level. Therefore, the curve drawn through the mean values in each case is an approximation of the true regression. Let us examine the meaning of the curve in the case of 7/2. As the maxilloalveolar breadth is increasing from 4 to 8 mm., the ratio of mid-sagittal alveolar length to the length of the anterior alveolar ridge is decreasing at a rapid rate. In other words, the former measurement is accelerating at a relatively slower rate than is the latter. However,

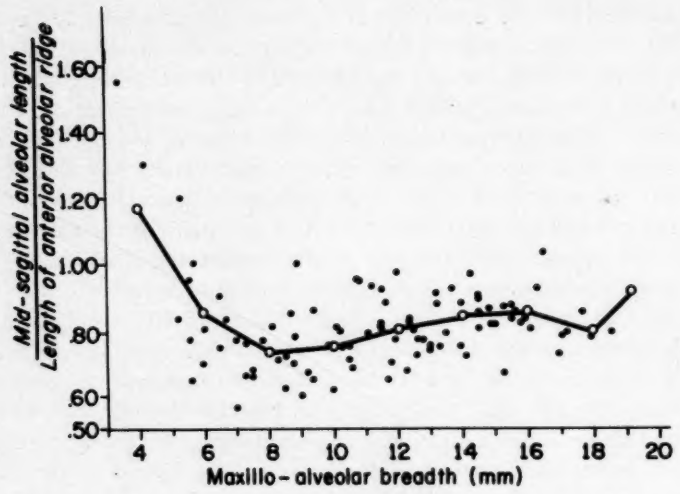

FIG. 11*A*

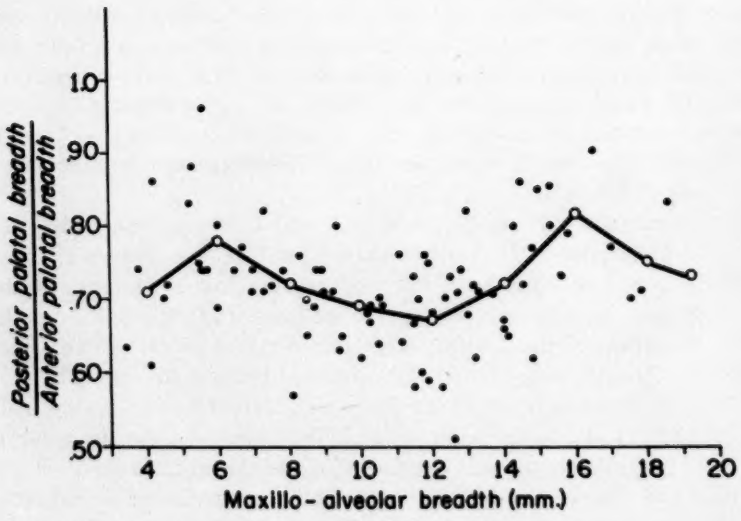

FIG. 11*B*

FIG. 11.—Values of four palatal indexes plotted against maxilloalveolar breadth to illustrate curvilinear regression. *A*, regression of index 7/2; *B*, regression of index 6/4; *C*, regression of index 2/4; *D*, regression of index 1/8.

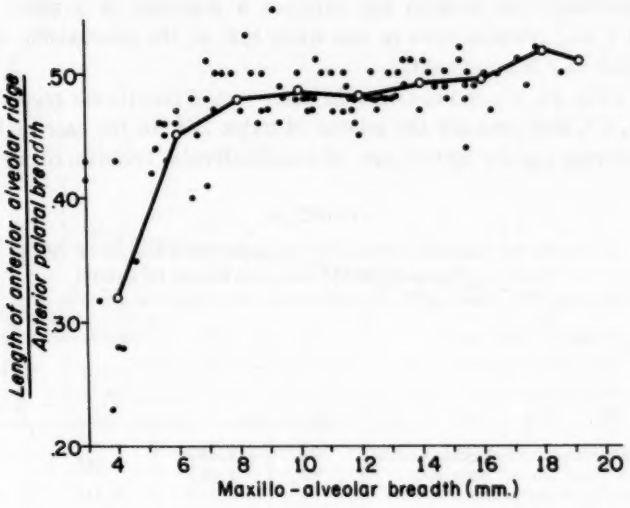

FIG. 11*C*

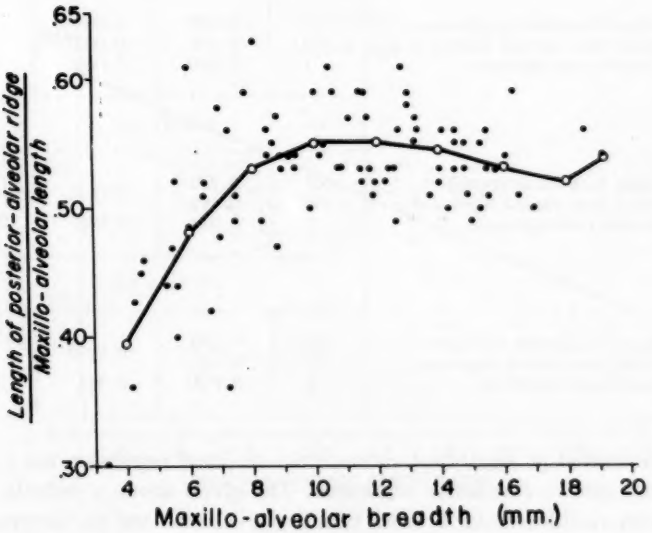

FIG. 11*D*

FIG. 11.—*Continued*

after the maxilloalveolar breadth has attained a diameter of 9 mm., the ratio of measurement 7 to 2 remains more or less fixed, that is, the relationship of the rate of gain of 7 to that of 2 is a constant.

Index 1/8 (Fig. 11, *D*) shows much the same sort of curvilinear regression as index 2/4 (Fig. 11, *C*), and both are the reverse of index 7/2. In the case of 1/8 and 2/4, the indexes increase rapidly up to 8 mm. of maxilloalveolar breadth, then both level off.

TABLE 3

ANALYSIS OF VARIANCE FOR CURVILINEAR REGRESSION OF FOUR PALATAL INDEXES ON MAXILLOALVEOLAR BREADTH

SOURCE OF VARIATION	D.F.	Sx^2	MEAN SQUARE	F
	Index 7/2			
Deviations from linear regression	93	1.88	0.018	1.0
Deviations from curved regression	92	1.66		
Curvilinearity of regression	1	0.213	0.213	11.8
	Index 6/4			
Deviations from linear regression	83	0.528	0.0064	1.0
Deviations from curved regression	82	0.468	0.0057	
Curvilinearity of regression	1	0.060	0.060	10.6
	Index 2/4			
Deviations from linear regression	101	0.230	0.0019	1.0
Deviations from curved regression	100	0.192		
Curvilinearity of regression	1	0.038	0.038	20.2
	Index 1/8			
Deviations from linear regression	88	0.230	0.0019	1.0
Deviations from curved regression	87	0.166		
Curvilinearity of regression	1	0.064	0.064	33.9

Index 6/4 showed no significant variance due to linear regression but a high degree of significance due to curvilinear regression. The graph shows a definite decrease in the index from maxilloalveolar breadth values of 6–12 mm. and an increase thereafter. We may discount both ends of the line because of small sampling values.

Of the remaining four indexes—2/1, 8 — 2/1, 8 — 7/4, 1/4, and 4/5—linear regression is shown to be a significant cause of variance (Table 2). Indexes 2/1 (Fig. 12, *A*) and 4/5 (Fig. 12, *B*) decrease at a constant rate, the latter more rapidly than the former. Indexes 8 — 7/4 (Fig. 12, *C*) and 1/4 (Fig. 12, *D*) both increase at constant and similar rates.

From the data presented herein the reader is at liberty to examine in detail the nature of the dimensional changes that occur as the palatal structures increase in size.

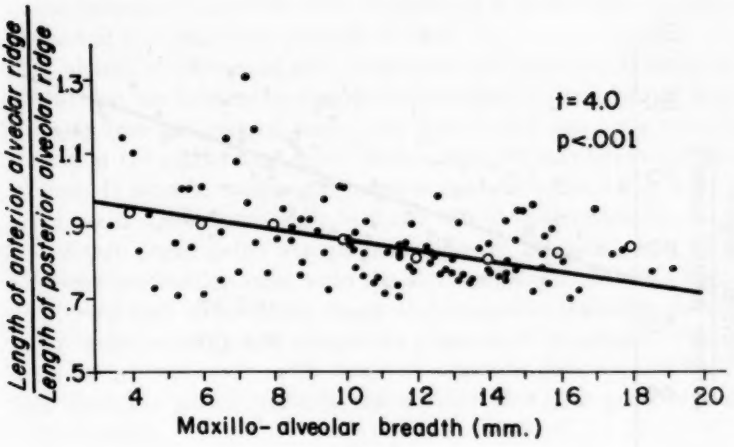

FIG. 12*A*

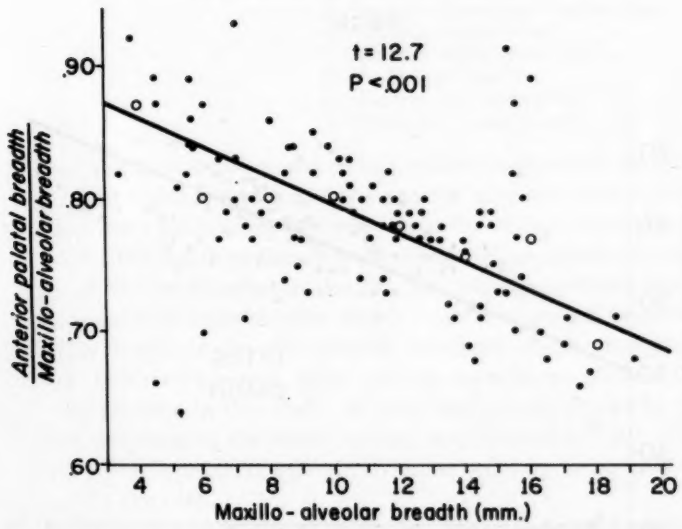

FIG. 12*B*

FIG. 12.—Values of four palatal indexes plotted against maxilloalveolar breadth to illustrate linear regression. *A*, regression of index 2/1; *B*, regression of index 4/5; *C*, regression of index 8–7/4; *D*, regression of index 1/4.

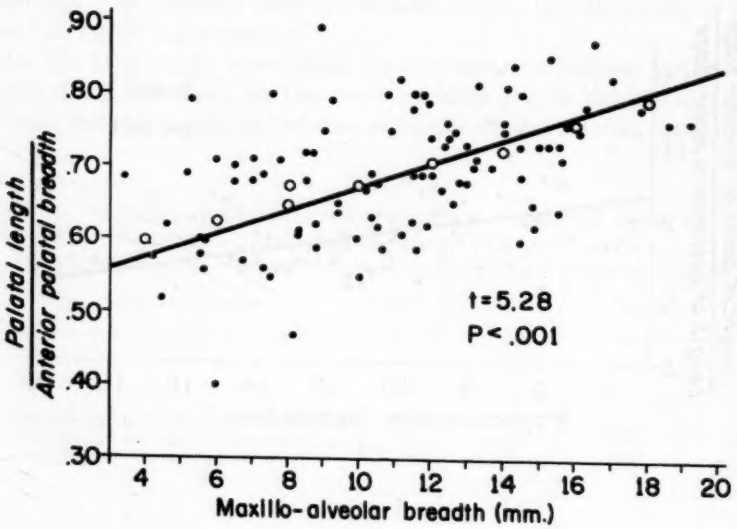

FIG. 12*C*

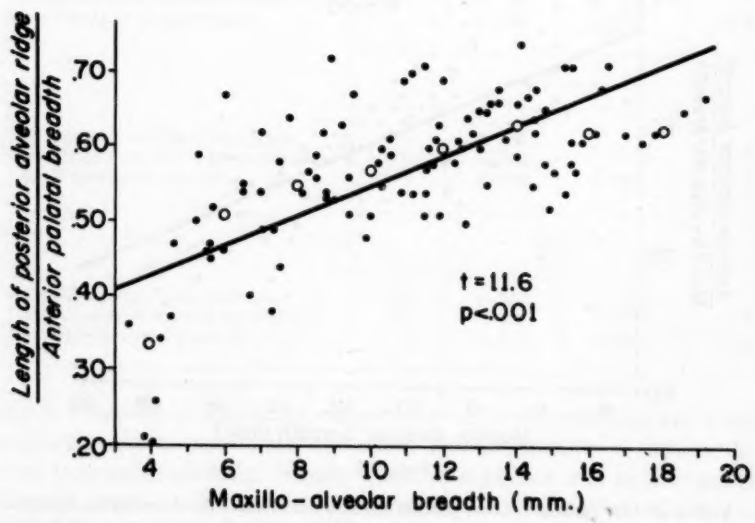

FIG. 12*D*

FIG. 12.—*Continued*

Caution must be exercised in the interpretation of these changes, since indicial changes are related to increase in maxilloalveolar breadth and it is extremely unlikely that the latter increases at a constant rate per unit of time.

A general picture of differential rates of increase now emerges. At the same time, it seems evident that the increase in maxilloalveolar breadth from 3 to 18 mm. may be divided roughly into two unequal parts—one from 3 to 8 mm.; the other from 8 to 18 mm. Many of the acceleration ratios change after the maxilloalveolar breadth has exceeded 8 mm. It must be emphasized, however, that the figure 8 mm. is by no means an exact one, nor do the authors imply by it any sort of "interphase."[13]

In the 3–8-mm. segment the various dimensions of the palate can be ranked in order of relative acceleration rates as in the accompanying table. This may be interpreted as showing that in the earlier stages of development the entire alveolar arch is growing at a faster rate in length than is the palate itself in breadth. The manner in which the palate increases in breadth, however, cannot be demonstrated by this study. In general, then, the palate and adjacent alveolar structures grow in length more rapidly than in width.

Acceleration Rank	Measurement
1	1—Length of posterior alveolar ridge
2	2—Length of anterior alveolar ridge
3	8—Maxilloalveolar length
	7—Mid-sagittal alveolar length
	8—7—Palatal length
4	4—Anterior palatal breadth
5	5—Posterior palatal breadth

In the later stages of development certain palatal proportions tend to become more or less fixed. The width and length of the anterior alveolar segment tend to return to an 85 per cent ratio (width/length), whereas in the earliest stage the ratio was close to 120 per cent. The length of the posterior alveolar ridge appears to level off at about 52 per cent of the maxilloalveolar length. The same proportion is reached by the relationship of anterior alveolar ridge length to anterior palatal breadth. The ratio of maxilloalveolar length to breadth remains relatively stable throughout the period considered at about 84 per cent. Since the conventional maxilloalveolar index is the reverse of the one used in this study, we may compare the fetal with the adult index by taking the reciprocal of the former, giving us a value of 119 per cent. In the adult this would be a brachyuranic or exceedingly broad arch, the mean adult value in whites being about 112 per cent.

It is interesting to compare fetal and adult palatal indexes. Conventionally in anthropometry this index is the relationship of palatal breadth to palatal length. In white adults the mean value is around 82 per cent. In fetuses this relationship begins with 167 per cent and decreases to 125 per cent. This decrease is achieved by a *constantly* increasing rate of acceleration of palatal length relative to that of palatal breadth. It does not necessarily follow that the decreasing ratio of acceleration rates observed in the period under study continues to be constant thereafter.

There is little point and insufficient space to continue with these detailed interpretations. The data presented herein permit the reader to pursue these himself if he so desires. The conclusions seem to be rather obvious: (1) the proportions of the

bony palate do not remain fixed throughout the period of growth (7–18 weeks) either in terms of over-all dimensional relationships or in structural interrelationships; and (2) the various dimensions of the palate increase at changing rates of acceleration which bear definite and predictable relationships to one another. Growth of the bony palate is not a haphazard affair but is a carefully co-ordinated and integrated pattern of accelerating and decelerating growth of the many constituent bony structures. Keith and Campion[14] wrote of the "ingenious and elaborate mechanism which underlies growth of the face as a whole" and referred morphologic irregularities and pathologic conditions of the face to arrests or disturbances of "this elaborate machinery." Keith and Campion studied postnatal growth. In the present paper an attempt was made to show how elaborate this "machinery" is in the course of prenatal development. Departures from the normal patterns will be more easily detected, and the search for arresting or disturbing factors may then begin at an earlier ontogenetic level of development.

As elsewhere throughout the body of the growing fetus, the ossification of the bony palate and surrounding alveolar structures follows a pattern that exemplifies the principles of allometry.

SUMMARY

There has been scant and inadequate study of the changing morphologic relationships achieved by the premaxillae, maxillae, and palatine bones as they develop from ossification centers to the eventual formation of the bony palate as known in postnatal life. In an attempt to describe the normal morphologic sequence of events that take place, a series of 151 fetuses ranging in age from 7 to 18 weeks was cleared and stained with alizarin red S. Study of the stained bony palate was conducted by microscopic examination, by palpation, by measurement, and by photography. It was found that eight morphologic stages in development could be described. Allometric studies indicated that the bony palate achieves its normal, characteristically human proportions through rigidly controlled, though highly complex, patterns of acceleration and deceleration of growth in the various bony components of the palate, that is, through differential growth of the component parts.

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The Relationship between Acid Production and Enamel Decalcification in Salivary Fermentations of Carbohydrate Foodstuffs

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It is generally accepted that carbohydrate foodstuffs retained in the mouth and around the teeth provide the substrate for the bacterial action which results in the formation of the acids associated with dental caries. It is also recognized that some carbohydrate foodstuffs may be more important than others in this respect. Previous workers have suggested that the greater the amount of acid produced by a food during fermentation, the greater might be the effect on enamel. Thus Bibby, Goldberg, and Chen[1] used the acid-production property (along with a food-retention factor) to obtain decalcification potentials of a large variety of foods. Later Ludwig and Bibby[2] found that some foods having a high decalcification potential produced lower plaque pH values than did foods having a low decalcification potential. However, it has not been shown whether foods producing large amounts of acid actually have the power to cause greater enamel decalcification than foods producing less acid.

In other experiments the decalcification of enamel in food-saliva mixtures has been studied but not related to the amount of acid produced by the foods. For example, Osborn, Noriskin, and Staz[3] examined macroscopically the surfaces of whole, noncarious teeth for signs of decalcification, while Jenkins, Forster, Speirs, and Kleinberg[4, 5] measured the increase of calcium and phosphorus in mixtures containing powdered enamel and later substituted calcium phosphate for the powdered enamel. Both these groups of workers found differences in enamel decalcification after incubating crude and refined carbohydrates in saliva which could not be explained on the basis of pH. However, in neither study was an actual titration made of the acid produced by the food-saliva mixtures, so that no relationship could be seen between total acid production and enamel decalcification.

Thus some previous workers found differences in the amounts of acid produced by various foods in saliva but did not relate these to effects on enamel, while others showed differences in enamel decalcification but did not relate them to differences in acid production. The primary purpose of the present study, therefore, was to demonstrate whether there exists a direct relationship between the amount of acid produced by salivary fermentation of carbohydrate foods and the amount of accompanying enamel decalcification.

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EXPERIMENTAL METHODS

The method used in this investigation was designed to allow better quantitation of results than was possible in Osborn's experiments[3] and, at the same time, to avoid the various experimental complications which Jenkins *et al.* encountered with their method.[4, 5] The procedure consisted essentially of suspending blocks of enamel in the various food-saliva mixtures, measuring the acid produced during the experimental period by titration, and taking the enamel weight loss as a measure of the accompanying decalcification. Permanent central incisor yearling bovine teeth were used as the enamel source. These teeth have definite advantages, because of their ready availability, their complete freedom from caries, and their large size, which makes it possible to obtain fairly large blocks of enamel.

To obtain the test blocks, the crowns of the bovine teeth were cut longitudinally, and all the underlying dentin was carefully removed. The enamel was then cut into blocks by a sectioning machine with a calibrated moving platform,[6] so that all the test blocks were uniform in shape and size. From each tooth about six enamel blocks were obtained, each measuring approximately 7 by 4 mm., having a thickness of approximately 1 mm., and weighing between 70 and 80 mg. A small hole was drilled through each block with a No. 1/2 carbide bur. Finally, the blocks were polished lightly on all surfaces with fine silicon carbide paper (which removed any remnants of dentin), washed, and dried to constant weight before use.

To prepare the foods for fermentation tests, 10 gm. of each food were blended for 5 minutes with 100 ml. distilled water in a blender.* Two 30-ml. samples of this 10 per cent food suspension were transferred to two 50-ml. flasks. To each flask was added 5 ml. of pooled, paraffin-stimulated whole saliva, and the pH of each mixture was measured.†

One enamel block was suspended in each mixture by looping one end of a fine nichrome wire through the previously prepared hole, while the other end passed through a cork stopper and was supported there with wax. After 6 hours' incubation and agitation in a 37° C. water bath, the suspended blocks were lifted out of their flasks, washed thoroughly in a stream of distilled water, removed from the wire, and placed in a 70° C. vacuum oven to dry to constant weight. The terminal pH of each mixture was measured, and the acid formed was determined by titrating back to the starting pH with 0.05 N sodium hydroxide.

On the basis of experience and weight-loss tests in a simple acid buffer, it was found that the same enamel blocks could be used up to four times, in the same or in different food mixtures, and still give reliable results. Before each re-use, however, all surfaces of the blocks were polished again on the fine paper, washed, and dried to their new contant weight.

Preliminary experiments indicated that different amounts of acid were produced by a given food with different saliva pools. To allow for this variation, foods were tested in groups, using the saliva pool collected on a particular day. Each group was run on 3 days, thus using three different saliva pools; furthermore, each food was always run in duplicate. In this way, six readings for both acid production and enamel weight loss

* Waring Blendor.

† A Beckman Zeromatic pH meter was used.

were obtained for each food. It was felt that even small differences in enamel weight loss from food to food were meaningful if reflected on each of the 3 days. Comparisons could be made of foods *within* a given group but not between foods of different groups, unless an adequate number of controls was carried over from one group to another.

RESULTS

The data in Table 1 give the enamel weight loss and acid production resulting from the fermentation of two groups of foods. Although the results showed some variation from day to day, the relative daily differences in acid production and enamel weight

TABLE 1

ENAMEL WEIGHT LOSS AND ACID PRODUCTION IN 6-HOUR SALIVARY FERMENTATIONS OF FIVE FOODS

Foods	Enamel Weight Loss (Mg.)			Titratable Acidity (Ml. 0.05 N NaOH)			pH Change		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
White bread	0.6 0.5	0.3 0.2	0.3 0.3	9.1 9.1	8.2 8.1	6.8 7.2	6.4–4.2	6.4–4.2	6.4–4.5
Mean	.....	0.4	.....	.....	8.4	.....			
Whole-wheat bread	0.4 0.2	0.1 0.1	0.2 0.2	12.4 12.5	11.2 10.3	9.5 10.0	6.3–4.2	6.3–4.3	6.3–4.5
Mean	.....	0.2	.....	.....	11.0	.....			
Corn flakes	0.4 0.2	0.6 0.8	0.6 0.6	3.9 3.5	4.3 4.4	4.8 5.0	6.5–4.6	6.4–4.2	6.5–4.2
Mean	.....	0 5	.....	.....	4.3	.....			
Wheat flakes	0.1 0.3	0.4 0.6	0.4 0 6	8.5 8.4	8.9 8.8	9.5 10 0	6.5–4.4	6.5–4.1	6.5–4.2
Mean	.....	0.4	.....	.....	9 0	.....			
Bran cereal	0.2 0.0	0.0 0.1	0.0 0.2	15.7 15.9	15.8 15.9	16.4 17.1	6.4–4.5	6.4–4.3	6.5–4.3
Mean	.....	0.1	.....	.....	16.2	.....			

loss between one food and another were maintained. Thus, on the first day, white bread produced less acid than did whole-wheat bread, but greater enamel weight loss, and these relationships were maintained on the second and third days. It was observed that white bread produced less acid but greater enamel loss than did whole-wheat bread on *each* of the 3 days, and the small differences were therefore accepted as being meaningful. Similar day-to-day relationships were shown by the second group, in which an interesting observation was the relatively low decalcification with bran cereal,* compared with corn flakes, although the bran cereal produced about four times as much acid. On the basis of these results and results from 23 other foods, it was concluded

* Kellogg's All-Bran.

that there was no direct correlation between the amount of acid produced by a food in saliva and the extent of the accompanying decalcification.

It can be noted from the results in Table 1 that the means of the six readings of titratable acidity and enamel weight loss reflect the general daily differences between the foods. In the following illustrations, therefore, for purposes of clearer presentation, the data are given in the form of bar graphs (Figs. 1–5), showing the mean values of titratable acidity and enamel weight loss for each food.

Figure 1 shows the acid formation and enamel decalcification resulting from the fermentation of different fractions of wheat grains. A lack of correlation between acid production and enamel decalcification was again shown. Refined white flour (from which germ and bran fractions had been removed) was associated with a relatively

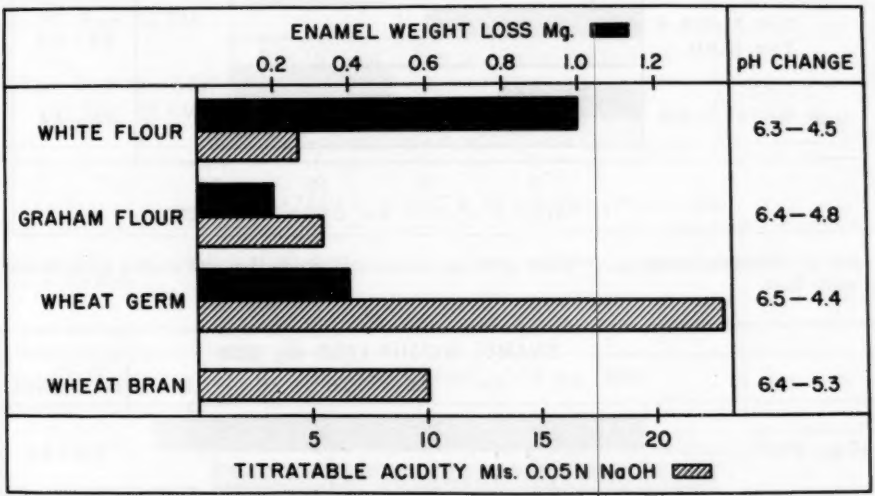

FIG. 1.—Enamel decalcification and acid production in 6-hour salivary fermentations of two wheat flours and wheat grain fractions.

high enamel weight loss, while the unrefined Graham flour (containing both germ and bran) was responsible for a comparatively small enamel weight loss, although it produced more acid. This, as well as the low weight-loss figures associated with germ and bran alone (despite greatly raised acid formation), suggested that bran and germ might contain factors which in some way protected the enamel from decalcification. This evidence was supported by subsequent experiments in which additions of germ and bran were made to white flour. The results of these experiments are shown in Figures 2 and 3 and show reduced enamel weight loss in the presence of these additions, even though the acid production is generally raised.

The next series of experiments was conducted to show whether the protective properties were shown by the inorganic constituents of wheat germ and bran. These were ashed at 500° C., and the ash of each was added to white flour in amounts of ash corresponding to the proportions of germ and bran added in the previous experiment (Figs. 2 and 3). It was clear from the results (Figs. 4 and 5) that the ash of both germ and bran was capable of producing approximately the same reduction in enamel solu-

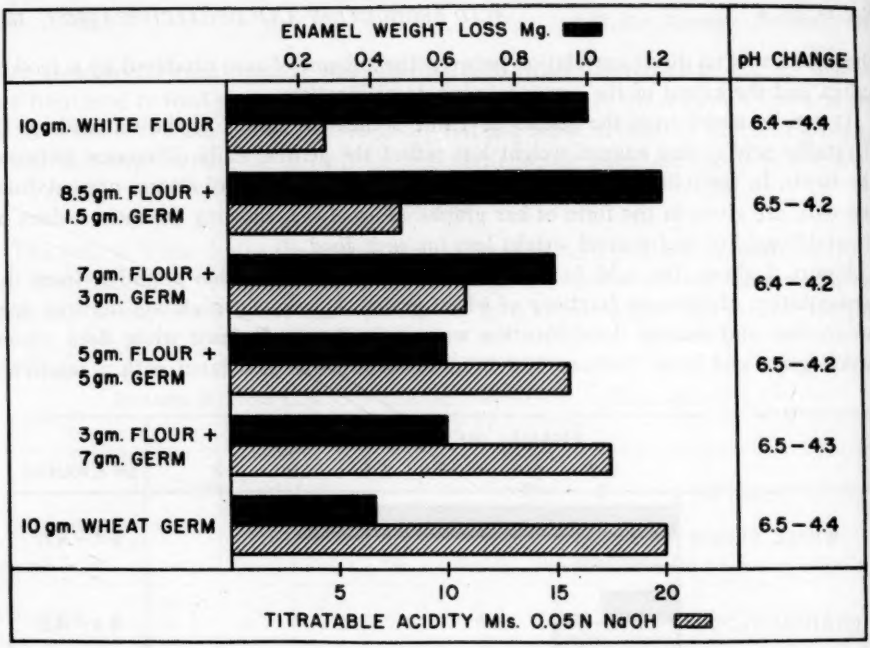

FIG. 2.—Effects of additions of wheat germ on 6-hour acid production and enamel decalcification of white flour.

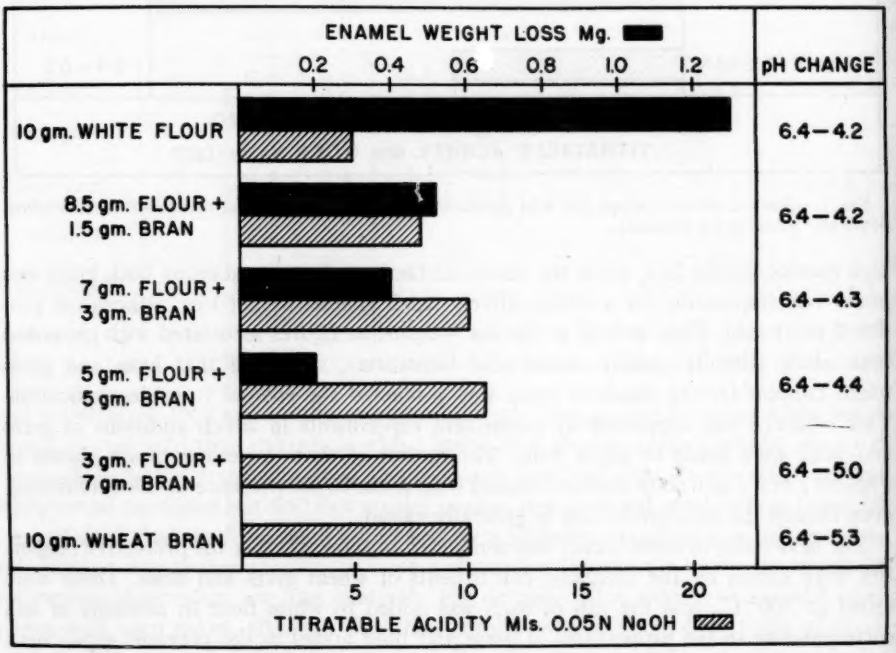

FIG. 3.—Effects of additions of wheat bran on 6-hour acid production and enamel decalcification of white flour.

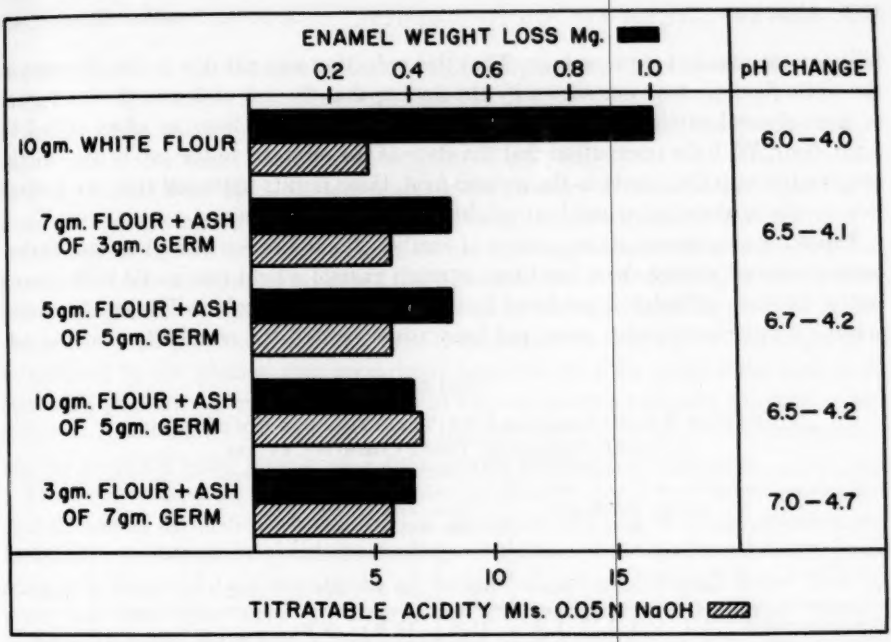

FIG. 4.—Effects of additions of ashed wheat germ on 6-hour acid production and enamel decalcification of white flour.

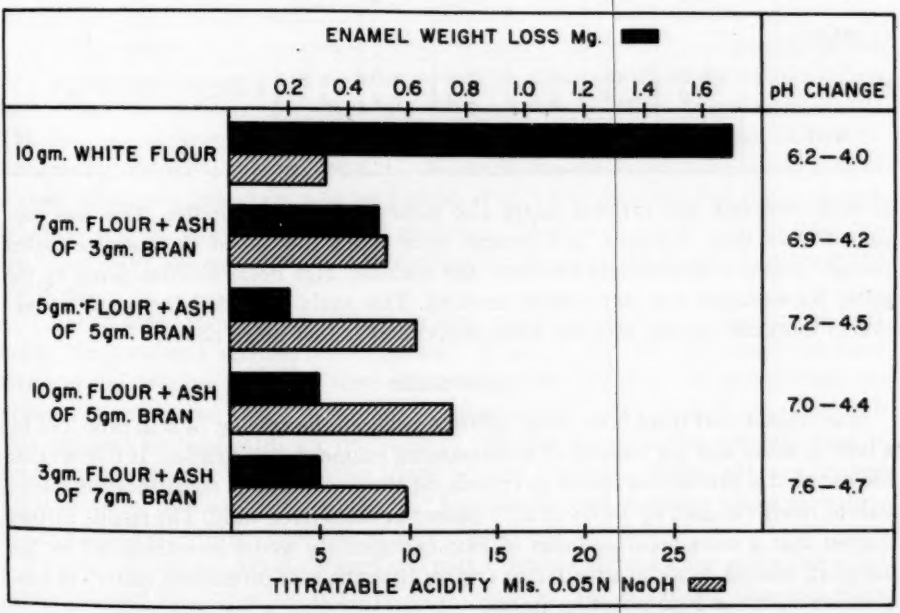

FIG. 5.—Effects of additions of ashed wheat bran on 6-hour acid production and enamel decalcification of white flour.

bility as the unashed germ or bran. That this reduction was not due to the decrease in the white-flour content was shown by the finding that the ash of 5 gm. of wheat germ or bran showed similar effects when added to 10 gm. white flour as when added to 5 gm. flour. With the reservation that the state of the minerals in the ash is not strictly comparable with their state in the natural food, these results indicated that the protective agents in wheat germ and bran might be inorganic in nature.

Table 2 shows the results of a group of candies. It can be seen that chocolate-coated coconut cream* caused about ten times as much enamel weight loss as did milk chocolate or caramel, although it produced less acid than milk chocolate. The results of the preceding experiments with germ and bran ash suggested the possibility that the ash

TABLE 2

ENAMEL WEIGHT LOSS AND ACID PRODUCTION IN 6-HOUR SALIVARY FERMENTATIONS OF THREE COMMON CANDIES

Candy	Enamel Weight Loss (Mg.)			Titratable Acidity (Ml. 0.05 N NaOH)			pH Change		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Chocolate-coated coconut cream	1.1 1.0	1.3 1.1	1.0 0.9	11.6 11.8	12.0 11.8	9.8 9.8	6.8–4.0	6.7–4.0	6.5–4.1
Mean	.....	1.1	.....	.....	11.1	.....			
Milk chocolate	0.2 0.2	0.3 0.0	0.0 0.0	13.3 13.2	14.0 13.0	12.8 12.9	6.7–4.2	6.7–4.2	6.7–4.2
Mean	.....	0.1	.....	.....	13.2	.....			
Caramel	0.0 0.0	0.0 0.0	0.1 0.1	7.8 7.9	8.0 7.9	5.5 5.3	6.6–4.3	6.8–4 3	6.8–4.9
Mean	.....	0.0	.....	.....	7.1	.....			

of milk chocolate and caramel might also show protective properties. This was confirmed when milk chocolate and caramel were ashed and added to chocolate-coated coconut cream in appropriate amounts; the normally high decalcification given by the latter fermentation was significantly reduced. This again suggested the protective action of inorganic agents, with the same reservation as that noted above.

DISCUSSION

It is evident that there is no direct relation between the amount of acid produced by a food in saliva and the amount of accompanying enamel decalcification. It follows that the use of acid-production figures as criteria for the assessment of decalcification potentials of foods (as used by Bibby *et al.*[1]) cannot be considered valid. The results further suggest that a more accurate index of food cariogenicity would be established by the use of an enamel decalcification factor (rather than the acid-production factor) in conjunction with the food-retention factor.

There also seems to be no direct relation between the terminal pH of the mixtures

* Peter Paul Mounds.

and the amount of enamel decalcification. Although, in some of the results, low enamel weight-loss figures were associated with relatively high terminal pH readings (Figs. 1, 3, and 5), this was by no means a regular finding. For example, low decalcification values with bran cereal (Table 1), wheat germ (Figs. 1 and 2), and the small additions of bran to white flour (Fig. 3) were found in the presence of a low pH, comparable to those of foods giving high enamel decalcification in the corresponding groups.

One way of explaining the low enamel decalcification produced by some foods in the presence of large amounts of acids and low pH conditions is by thinking of the foods as containing protective agents which are capable of protecting enamel against the action of the acid. Such protective agents appear to be present in wheat germ and bran. This is indicated by the findings that germ, bran, and Graham flour (containing both germ and bran) give low enamel decalcification in association with high acid production and is further supported by the fact that, after the removal of germ and bran during refining, the resultant white flour gives a considerably greater decalcification.

These observations bear out the contention of Osborn *et al.*[3] that factors protective against enamel decalcification may be lost during the refining of crude carbohydrate foods. They are also in line with the findings of Jenkins *et al.*[4] that less calcium phosphate was dissolved by fermentation of "brown" flour and "brown" bread than by white flour and white bread. In experiments with cotton rats, Taketa and Phillips[7] and Constant, Sievert, Phillips, and Elvehjem[8] have found oat hulls to be anticariogenic in the diets of cotton rats. The hull is the same portion of the oat grain as the bran is of the wheat grain. Constant *et al.*[8] showed that whole oats were of low cariogenicity but that, following the removal of the hulls, the resultant groats and rolled oats were of high cariogenicity.

As to the nature of the postulated protective agents, the present study suggests that they are inorganic in nature. This is supported by a number of animal studies which have shown that the mineral content of a diet can markedly affect its cariogenicity.[8–14] This does not exclude the possibility of the coexistence of organic protective factors, evidence for which has been presented by Osborn,[15] who concluded that calcium salts of organic acids were important, and by Jenkins *et al.*,[4] who demonstrated the protective action of organic phosphates, notably phytates. Taketa and Phillips[7] found that the ash of oat hulls was not effective in controlling the development of dental caries in the cotton rat.

It may be noted from the results that, in some mixtures attaining similar terminal pH levels, the titratable acidities varied greatly (Table 1, Fig. 2). Conversely, in some mixtures the terminal pH levels were different, yet the titratable acid present was approximately the same (Figs. 3, 4, and 5). These observations suggest the possibility that different kinds of acids are produced by different foods. If this were the case, it could explain the lack of relationship found between titratable acidity, pH, and enamel decalcification, because several studies[16–19] have shown that there are broad variations in the enamel-dissolving capacities of different organic acids.

The finding that the ash of caramel and milk chocolate exerted some protective effect against enamel decalcification requires some comment. The possibility that this effect is the result of the appreciable content of milk products in these candies is suggested by the finding of Shaw, Ensfield, and Wallman[20] that the addition of milk products reduced the cariogenicity of animal diets.

The present study, in drawing attention to the lack of correlation between acid production and enamel decalcification, demonstrates the existence of protective properties in some foods, which is another factor to be kept in mind in any attempts to establish the relative cariogenicity of different foods.

SUMMARY

Using titration with 0.05 N NaOH and blocks of bovine enamel, acid production and enamel decalcification were compared in food-saliva mixtures.

A lack of correlation was found between the two measurements, and evidence was presented to suggest that Graham flour, wheat germ, and wheat bran contain factors which protected the enamel from decalcification.

When the ash of wheat germ and bran were added to white flour, acid production was increased, but enamel decalcification was reduced. The ash of caramel and milk chocolate also reduced the decalcification produced by another candy.

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The Thromboplastic Activity of Human Saliva

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The purpose of this study is to determine the effect of saliva in hastening blood coagulation and to establish the nature of the accelerator factors present in human saliva. The observation that saliva decreases the clotting time of whole blood is not new, but the mechanism and mode of action has not been well described.[1, 2, 3] According to the current theory of blood coagulation,[4, 5] there are three phases in the clotting of blood (Fig. 1). The initial phase is the formation of complete thromboplastin. Next comes the conversion of prothrombin to thrombin by thromboplastin, and, finally, there is a conversion of fibrinogen to fibrin by thrombin.

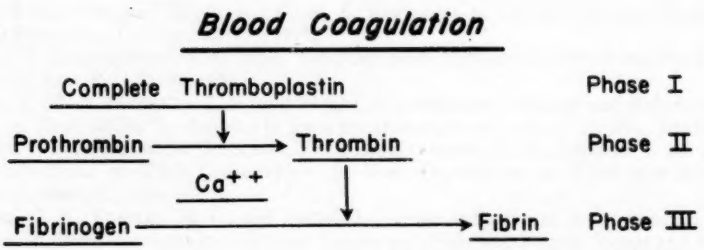

FIG. 1.—The three phases of blood coagulation

The formation of complete thromboplastin (or prothrombin activators or prothrombinase) may occur* (A) from the blood plasma when the plasma factors in the presence of platelets and calcium interact and form a complete thromboplastin (Fig. 2); (B) from the tissues, which act as incomplete thromboplastin and require several other plasma factors to be added—labile (V), proconvertin (VII), Stuart (X) factors—and calcium to form a complete thromboplastin (Fig. 2); (C) Russell's viper venom also acts as incomplete thromboplastin and requires labile (V), Stuart (X) factors, and phospholipids (serine or ethanolamine-phosphatids in plasma or in platelets) and calcium to form complete thromboplastin.

EXPERIMENTAL METHODS

Whole mixed saliva was collected in sterile bottles from each subject, and part was centrifuged at 1,500 rpm for 20 minutes for the preparation of whole supernatant. The

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Presented at the thirty-eighth general meeting of the International Association for Dental Research in Chicago, March, 1960, and awarded Honorable Mention in the Edward H. Hatton Award Competition.

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* The terminology for the blood factors is adapted from the International Committee on Nomenclature of Blood Clotting Factors (*J.A.M.A.*, **170**:325, 1959, and *Ann. Int. Med.*, **51**:841, 1959).

sediment was washed twice with physiologic saline and brought up to the volume of the original saliva sample. Parotid saliva was collected with a Curby cup. Submaxillary and sublingual salivas were collected by direct cannulation of the Wharton's and Bartholin's ducts, respectively, or, as in a few cases, by using Schneyer's appliance. The amount of sublingual saliva necessary for our experiments was under 1 ml.

The following standard hematological tests were modified to demonstrate the presence of coagulation factors in saliva.

A. In the determination of the clotting time of whole blood using the silicone technique,[6] blood samples from 80 healthy subjects were mixed with whole mixed saliva in different proportions. The ratio of blood to whole mixed saliva used was 4:1, 2:1, and 1:1. Also, the comparative effect of whole mixed, submaxillary, and sublingual salivas on bloodclotting time was studied on 40 healthy subjects, using four parts blood and one part saliva. For controls physiologic saline was substituted for saliva.

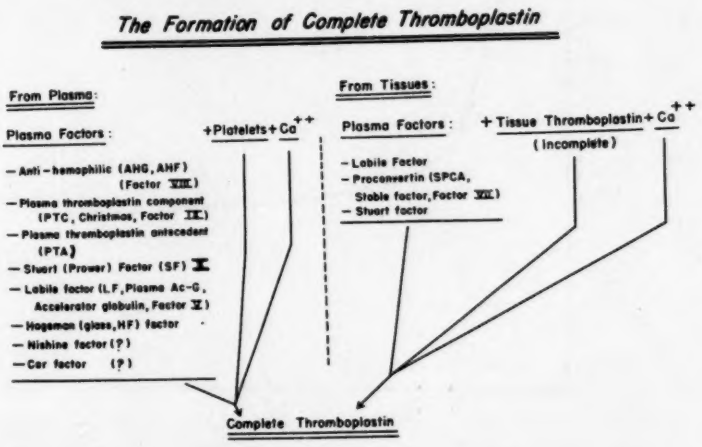
The Formation of Complete Thromboplastin
From Plasma:
Plasma Factors:
— Anti-hemophilic (AHG, AHF) (Factor VIII)
— Plasma thromboplastin component (PTC, Christmas, Factor IX)
— Plasma thromboplastin antecedent (PTA)
— Stuart (Prower) Factor (SF) X
— Labile factor (L.F, Plasma Ac-G, Accelerator globulin, Factor V)
— Hageman (glass, HF) factor
— Nishino factor (?)
— Cor factor (?)
+Platelets+Ca++
From Tissues:
Plasma Factors:
— Labile Factor
— Proconvertin (SPCA, Stable factor, Factor VII)
— Stuart factor
+ Tissue Thromboplastin (Incomplete) + Ca++
Complete Thromboplastin

FIG. 2.—The formation of complete thromboplastin from plasma and tissues

B. In the one-stage prothrombin time determination test, a commercial tissue thromboplastin is mixed with equal amounts of plasma and Ca^{++}. A firm clot forms in a short time, the time being dependent on the potency of the tissue thromboplastin used.[7]

By diluting the plasma with physiologic saline, a prothrombin dilution curve can be obtained (Fig. 3). The clotting time is increased by dilution. A similar effect is observed when the concentration of one or more of the following coagulation factors—prothrombin, fibrinogen, labile (V), proconvertin (VII), and Stuart (X)—is decreased.

We modified the above test by replacing commercial tissue thromboplastin with saliva in 30 healthy subjects. The plasma was also diluted with saliva. The activity of whole mixed, whole sediment, whole supernatant, parotid, and submaxillary salivas was tested. As controls, physiologic saline was used to dilute plasma instead of saliva.

C. Complementary to this test was the partial thromboplastin time in which 0.1 ml. of citrated platelet-poor plasma and an equal amount of 1:10 chloroform-brain extract, acting as incomplete thromboplastin, were incubated at 37° C. for 5 minutes.[8] The

clotting time of this incubated mixture is obtained by adding 0.1 ml. of a 0.03 per cent calcium chloride solution. The average clotting time was 138 seconds.

This test was modified for study of salivary clotting factors. The chloroform-brain extract (CBE) was replaced in 20 healthy subjects by oxalated whole mixed, oxalated whole sediment, and oxalated whole supernatant salivas. Oxalate salivas were necessary to prevent the salivary calcium from initiating clotting during incubation. Controls consisted of physiologic saline, replacing chloroform-brain extract.

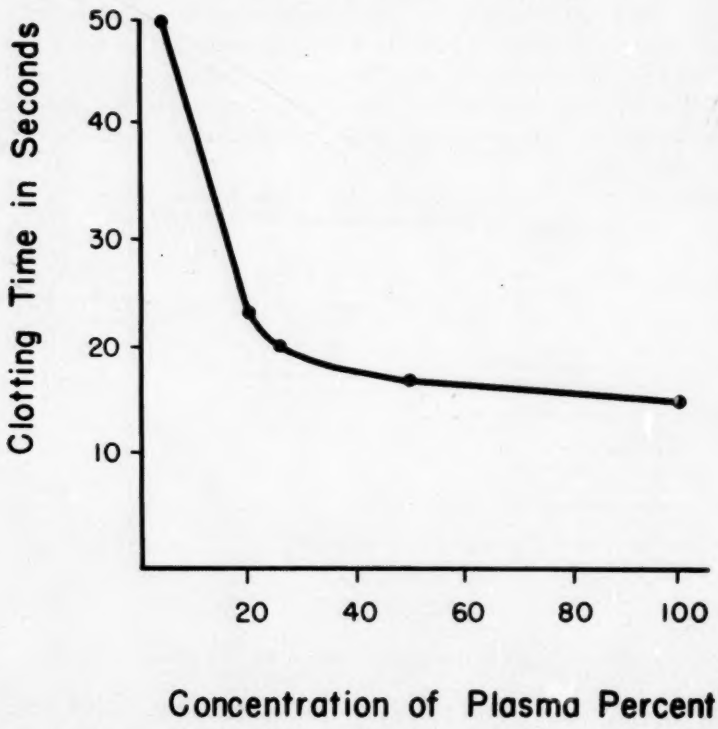

FIG. 3.—One-stage dilution curve from normal plasma, using Quick's rabbit brain preparation as thromboplastin.

D. Biggs, Douglas, and Macfarlane[9, 10] have demonstrated that three reagents prepared from normal blood—normal serum, normal plasma adsorbed with barium sulfate, and platelets—interreact in the presence of calcium to form a powerful thromboplastin. The mixture of the above-mentioned three reagents contain all the necessary blood-coagulation factors except prothrombin. The lack of any one of the blood-coagulation factors produces a coagulation defect, which is associated with abnormal thromboplastin formation.

These observations lead to the development of the thromboplastin generation test.[11, 12] This is a very sensitive test and facilitates the distinction between coagulation defects resulting from deficiencies in plasma, serum, or platelet activity. The object of this test is to measure the "intrinsic" thromboplastic activity of the blood.

By mixing the three normal reagents and incubating for 3–5 minutes, a powerful thromboplastin develops which clots the normal substrate plasma in 8–12 seconds. This preparation can be diluted 1:2, 1:4, 1:8, etc., with saline. The dilutions are tested with normal plasma substrate, and a curve relating clotting time and thromboplastin concentration can be drawn. Abnormal results are expressed as a percentage of normal activity (Fig. 4).

The localization of a defect in the coagulation is achieved by substituting, one at a time, into the normal system, the three fractions prepared from an unknown blood. For example, by substituting an unknown serum for the normal, it can be determined whether or not serum function in the unknown blood is normal or deficient.

We modified the thromboplastin generation test by replacing the three normal reagents—serum, adsorbed plasma, and platelets—one at a time, with whole mixed, whole supernatant, whole sediment, parotid, submaxillary, and sublingual salivas. The test was carried out on 30 healthy subjects following the same technique as in blood.

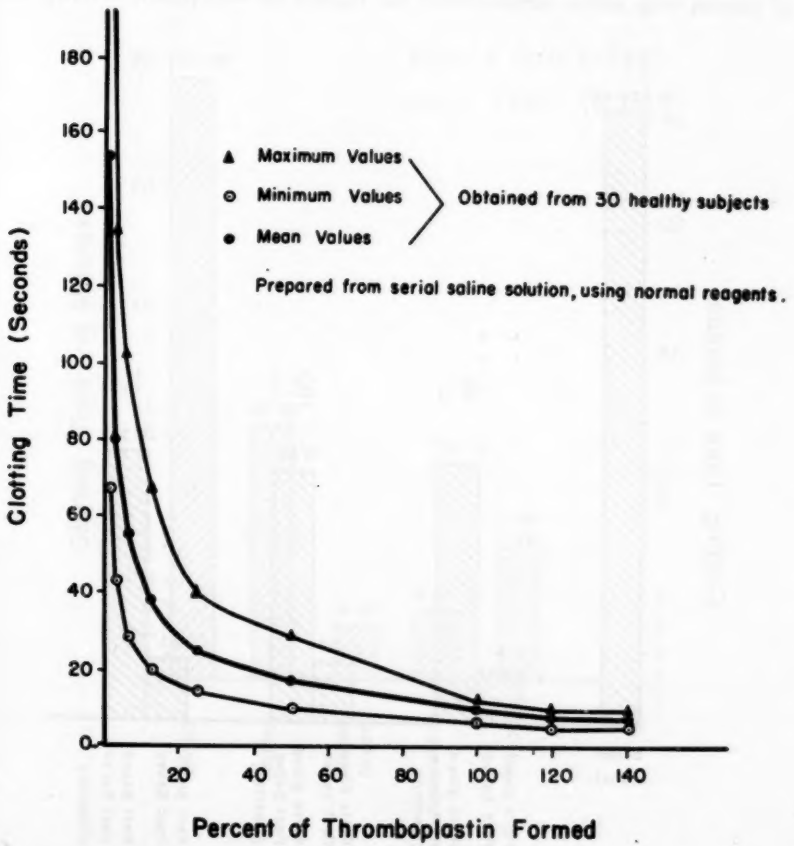

FIG. 4.—Thromboplastin dilution curve, for the calculation of the results of the thromboplastin generation test.

Dilutions were again made and tested with normal substrate plasma, and the thromboplastic activity was calculated from the individual dilution curves (Fig. 4).

RESULTS

A. *The effect of saliva on blood-clotting time.*—It was conclusively demonstrated that the saliva hastens the blood-clotting time. The blood-saliva ratio was not critical in decreasing the blood-clotting time. Dilutions from 4:1 to 1:1 exhibited a similar reduction in clotting time, with the 4:1 dilution being most effective (Fig. 5).

In the controls, it was shown that dilution of blood by saline hastened the clotting, but saliva was over four times more active than the control solutions.

It was also observed that the source of saliva affected the clotting time, whole saliva being most effective, followed by submaxillary and parotid (Fig. 6).

B. *The effect of saliva substituting for tissue thromboplastin in the one-stage prothrombin time.*—Saliva from all sources replaced the commercial tissue thromboplastin in the one-stage prothrombin time. It was found that dilutions of 20–50 per cent of plasma with saliva demonstrated the highest thromboplastic activity. Plasma

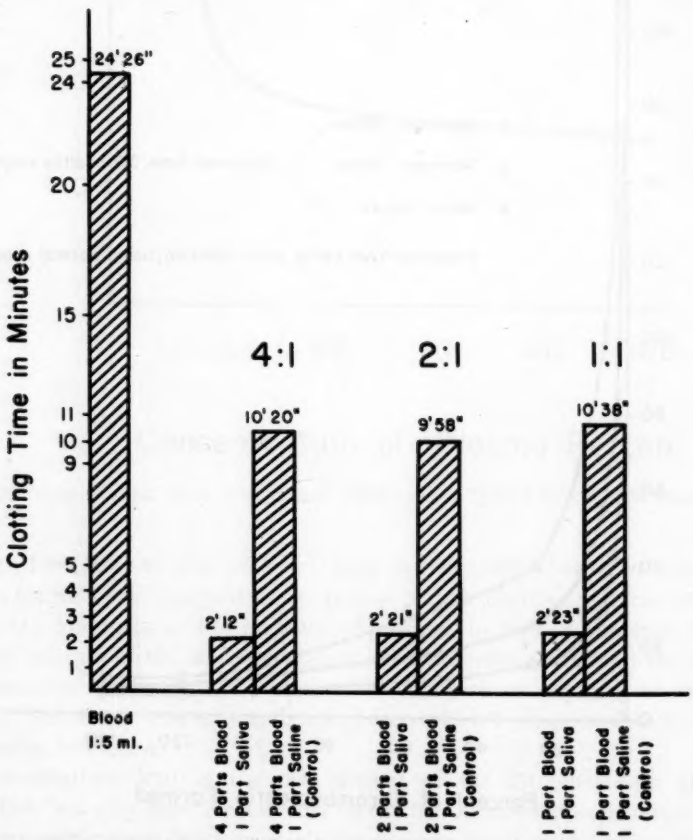

FIG. 5.—The effect of whole mixed saliva on blood-clotting time

concentrations below 20 per cent retarded clotting time because there was insufficient prothrombin and fibrinogen present, as these factors are supplied by plasma. Plasma concentrations above 50 per cent retarded clotting time because of insufficient salivary thromboplastin, as there was not enough saliva in the mixture (Fig. 7). When saliva was replaced entirely by saline, the plasma clotted very slowly; this corresponds to the so-called calcium clotting time of plasma.

The lengthening of the clotting time in increased dilutions is a manifestation of decreased concentration of prothrombin, fibrinogen, and labile (V), proconvertin (VII), and Stuart (X) factors.

C. *The effect of saliva substituting for chloroform-brain extract in the partial thromboplastin time.*—Oxalated whole mixed, oxalated whole supernatant, and oxalated sediment salivas were found to be about three times more active than the 1:10 dilution of chloroform-brain extract.

When oxalated saliva was diluted with saline, the clotting time was inversely proportional to the saliva concentration. Comparison of whole mixed saliva, whole super-

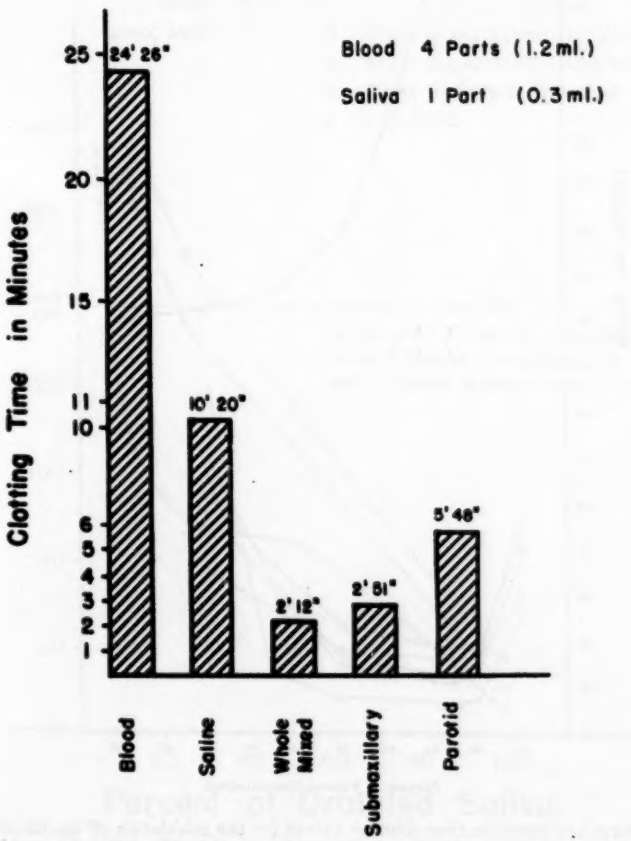

FIG. 6.—The comparative effect of whole mixed, submaxillary, and sublingual salivas on blood-clotting time.

natant, and whole sediment to chloroform-brain extract appear to have, respectively, 10, 3, and 2.5 times the activity of the chloroform-brain extract (Fig. 8).

D. *The effect of saliva substituting for serum, adsorbed plasma, and platelets in the thromboplastin generation test.*—Saliva from all sources completely replaced the factors contained in normal serum (Fig. 9).

Whole mixed saliva, its supernatant and sediment, as well as sublingual saliva, completely replaced the platelet factor. Parotid and submaxillary only partially replaced the platelet factor (Fig. 10).

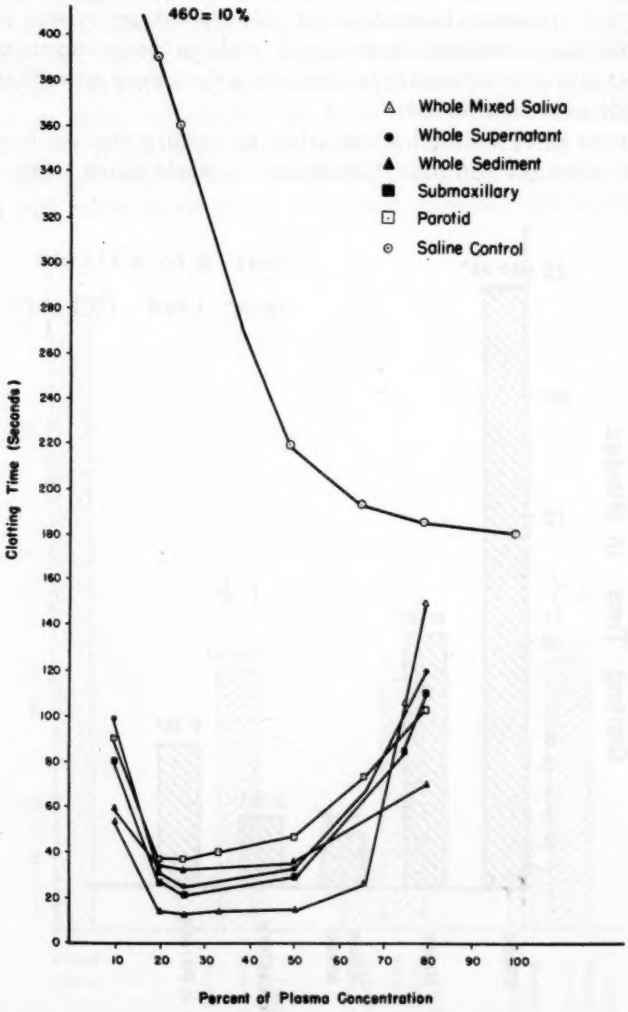

FIG. 7.—One-stage prothrombin time dilution curves for the calculation of the thromboplastic activity of saliva. Commercial tissue thromboplastin has been replaced by saliva. Normal fresh oxalated human plasma has been diluted with saliva.

On the other hand, parotid, submaxillary, and sublingual salivas failed to replace adsorbed factors, although whole mixed saliva showed some adsorbed plasma-like activity. This activity was found only in its sediment (Fig. 11).

Saline controls failed to demonstrate any activity at any level (Figs. 9, 10, 11).

DISCUSSION

The formed blood clot in the oral cavity usually contains some saliva. Knowledge of this fact led us to investigate the effect of saliva on the clotting mechanism and the nature of the blood-clotting accelerator factors previously noted in human saliva. The effect of saliva on decreasing blood-clotting is very dramatic.

Although whole blood-clotting time is a gross test and does not give detailed information of the intrinsic factors involved, it does reveal that the saliva must contain a certain factor or factors which are responsible for this dramatic reduction of the blood-clotting time.

Based on one-stage prothrombin, partial thromboplastin, and thromboplastin genera-

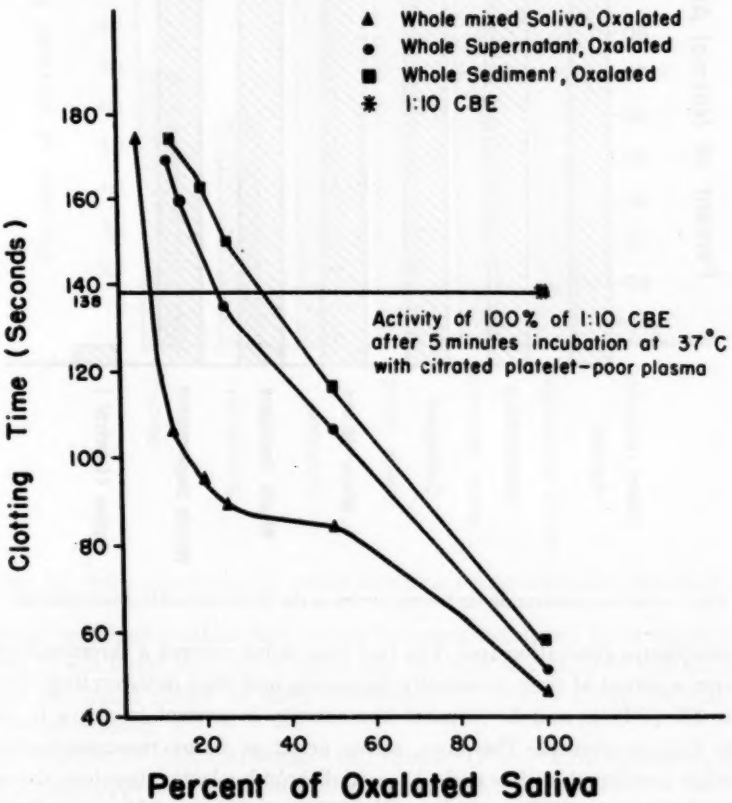

FIG. 8.—Comparison of diluted, oxalated saliva, and chloroform-brain extract in the partial thromboplastin test.

tion tests, we found that mixed saliva and the pure secretions of parotid, submaxillary, and sublingual glands all have thromboplastic activity similar to that found in tissue thromboplastin. This activity was much stronger than that found in the chloroform-brain extract. Table 1 shows the various coagulation factors which exist in the various blood fragments. From this table we can establish which thromboplastic factors exist in the saliva. Saliva from all sources completely replaced (*a*) commercial tissue thromboplastin in the one-stage prothrombin test, suggesting a lipo-protein like activity; (*b*) chloroform-brain extract in the partial thromboplastin test, indicating that saliva acts not only as a lipid but as a possible complex lipid and protein; and (*c*) serum in

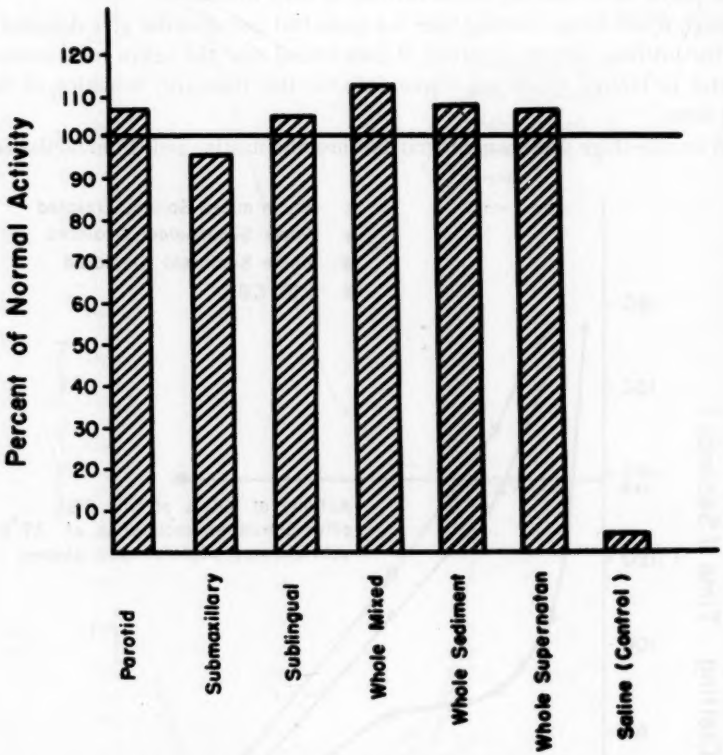

FIG. 9.—Saliva substituting for *normal serum* in the thromboplastin generation test

the thromboplastin generation test. The fact that saliva showed a thromboplastin generation over a period of time, constantly increasing and then deteriorating, shows that the factor IX (PTC)- and X (Stuart)-like activity is present in saliva in the form similar to that in plasma. Therefore, saliva acted as tissue thromboplastin in the (*a*) one-stage prothrombin time and (*b*) partial thromboplastin time test. Consequently, it can substitute for VIII (A.H.F.), IX (PTC), P.T.A., and Hageman factors and appears to have a lipid protein present.

The inability of salivary fluids to replace adsorbed plasma in the thromboplastin

generation test indicates that factor V (labile) is missing. However, some adsorbed plasma-like activity was observed in the sediment from whole mixed saliva.

The platelet-like activity of whole mixed saliva, found in both the sediment and the supernatant, appears to originate from the sublingual secretion and debris present.

SUMMARY

The effect of saliva on blood-clotting time was studied, and it was found that saliva decreases blood-clotting time. It was observed that the saliva-blood ratio was not critical in decreasing the clotting time.

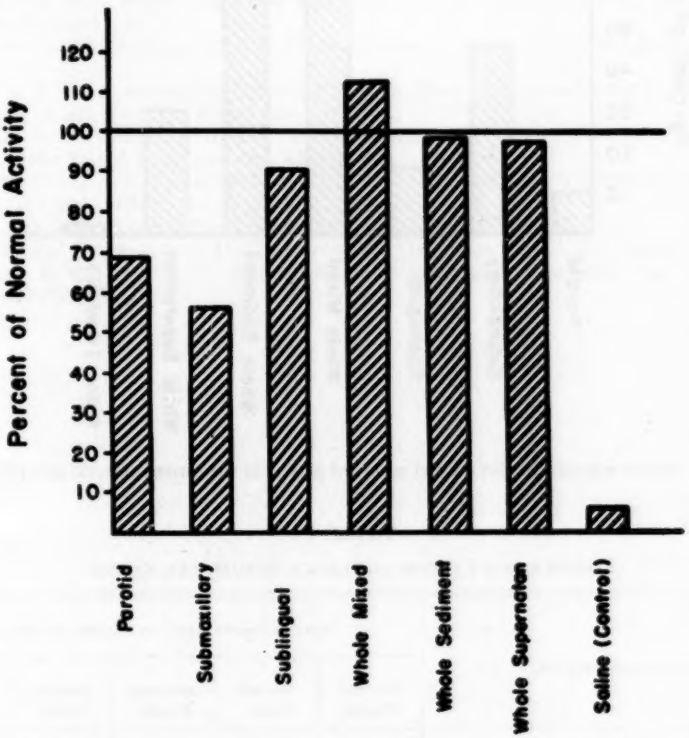

FIG. 10.—Saliva substituting for *normal platelets* in the thromboplastin generation test

Based on one-stage prothrombin, partial thromboplastin, and thromboplastin generation tests, it was found that mixed saliva and the pure secretions of parotid, submaxillary, and sublingual glands all have thromboplastic activity similar to that found in tissue thromboplastin.

Antihemophilic (VIII), Christmas (IX), Stuart (X), P.T.A., Hageman, and platelet-like activity appeared to be present in human saliva.

These results may have considerable clinical significance. In the first instance, demonstration of the presence of the different plasma thromboplastic factors in the saliva of normal persons may be of aid in developing diagnostic tests for patients with

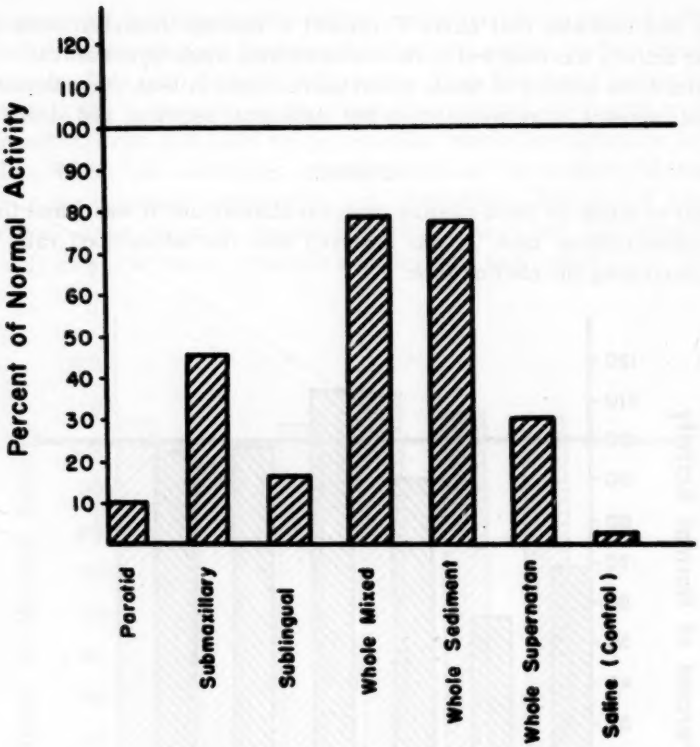

FIG. 11.—Saliva substituting for normal *adsorbed plasma* in the thromboplastin generation test

TABLE 1

COAGULATION FACTORS IN PLASMA, SERUM, AND SALIVA

COAGULATION FACTORS	VARIOUS PREPARATIONS OF PLASMA AND SERUM				
	Normal Plasma	Normal Serum	Adsorbed Plasma	Adsorbed Serum	Saliva
AHG-AHF-Factor VIII	+	0	+	0	+
PTC–Christmas Factor IX	+	+	0	0	+
PTA	+	+	+	+	+
Labile-Plasma Acc. Globulin-Ac-G–Factor V	+	0	+	0	0
Proconversion-Stable, SPCA–Factor VII	+	+	0	0	±
Stuart (Prower) Factor–SF	+	+	0	0	+
Hageman Factor–Glass-HF	+	+	+	+	+
Nishine–Factor(?)	+	0	+	0	?
Car–Factor(?)	+	+	0	0	?
Platelets	+	0	0	0	+
Prothrombin	+	0	0	0	0
Fibrinogen	+	0	+	0	0

blood dyscrasias. Second, these studies should contribute substantially to an understanding of the conditions influencing the clotting of blood in the oral cavity.

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Nerve Regeneration in Replanted Hamster Teeth

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Over the last sixty years a marked interest has developed in the possibilities of autogenous and homologous transplantation of teeth. Since 1945, many investigators have reported on transplantation and replantation of mature or develping teeth in animals and humans. Since pulps of transplanted developing teeth were not endodontically treated, this type of transplantation aroused renewed interest and speculation with regard to the importance of the pulp in transplant and replant procedures. Agnew[1] described nerve fibers in the 4-week tooth-germ transplants in Rhesus monkeys. The work of Bernick[2] and Apfel[3] indicated that these fibers were not regenerated nervous tissue, since the human tooth germ does not possess nerve fibers at this stage of development. Hale[4] suggested revascularization as essential for normal tooth development without resorption. Hammer,[5] in discussing tooth-germ transplantation, mentioned that the pulp in fully developed teeth could not survive when the teeth were transplanted. Pafford[6] emphasized the importance of revascularization of the dental pulp if degenerative processes were to be avoided. Myers and Nassembene,[7] the first to utilize the Syrian hamster in replantation studies, showed that apparently normal pulps with odontoblasts could be observed in replanted and transplanted teeth; however, no specific information concerning nerve regeneration was reported. Following apicoectomy of the teeth of monkeys, Butcher and Taylor[8] noted connective tissue invasion, osteoblastic deposition of osteoid tissue, and lack of odontoblastic regeneration within the pulp cavities. In a more recent investigation, Myers and Flanagan[9] reported that pulp regrowth occurred with "greater difficulty" than did regrowth of epithelium or periodontal membrane. They reported that the pulp seemed to contain the least capacity for regrowth.

Since nerve regeneration as an element of pupal regeneration in replanted teeth has not been previously reported, an investigation was undertaken to determine the possibility of regeneration of nervous tissue within the replanted mandibular second molar of the Syrian hamster.

MATERIALS AND METHODS

The mandibular second molars of ten Syrian hamster littermates of both sexes were utilized in this investigation. The animals were housed in wire-mesh cages and fed Purina Hamster Checkers. According to Orland,[10] the mandibular second molars of hamsters are usually erupted by the seventeenth or eighteenth day after birth, and the

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roots are completely formed within 36–38 days. The second molars were chosen because of eruption time, configuration, and suitability for splinting the replant to first and third molars, depending on the animal's age at the time of replantation.

Anesthesia from an intraperitoneal injection of veterinary nembutal* was attained within 2–4 minutes. The injection solution consisted of 1 ml. nembutal which had been diluted with 4.5 ml. of sterile distilled water, with 0.05 ml. of the solution administered for every 10 gm. of body weight. Accessibility to the oral cavity for the replantation procedure was accomplished by the use of the animal board modified after the table utilized by Costich.[11] An extraction forceps was fashioned from modified gold-foil tweezers. The beaks of the forceps were introduced into the root bifurcation, and the molar was extracted after careful luxation.

Twenty teeth were replanted in 10 animals, which ranged in age from 18 to 32 days. Replantation of the right and left molars was accomplished during the same surgical procedure. Most teeth were replanted immediately, but several were observed under a binocular dissecting microscope for periods of up to 5 minutes before replantation. Evidence of root fracture, underdeveloped apices, or remnants of attached periodontal membrane was noted and recorded for each replant. After replantation, half the replants were held in place by means of a self-curing acrylic splint placed over the erupted first molar and replant. The third molars on most animals were not erupted at the time of surgery.

Animals were sacrificed by chloroform inhalation at definite intervals to allow for gross and histologic examination of the replants. The mandibles were dissected, divided at the symphysis, and fixed in 10 per cent formalin. The mandibles of all the experimental animals were prepared for histologic examination regardless of the replant's retention or exfoliation, in order to maintain a complete histologic record of the individual replants. Decalcification was accomplished in equal parts of 20 per cent sodium citrate and 40 per cent formic acid for 14 days. The blocks were imbedded in paraffin and serially sectioned at thicknesses ranging from 5 to 7 μ. The Powers[12] modification of the Romane[13] method of silver impregnation, which clearly differentiated nerve fibers, was used.

RESULTS

At sacrifice the animals ranged in age from 34 to 63 days. Mature replants which had been retained from 16 to 43 days were available for histologic study. Sixteen of the twenty replanted teeth were observed to be in position. No replants which had been splinted with acrylic were lost, even though the acrylic splints were not retained until time of sacrifice.

Twelve of the sixteen retained teeth appeared clinically normal and showed no evidence of replantation procedures. Two replants exhibited increased mobility when compared with adjacent molars in the same quadrant. A dark discoloration was seen in two of the replants, one of which was in infra-occlusion and slightly lingual to the rest of the dental arch.

Apparent periodontal reattachment was observed in all retained replants, and the histologic appearance of these newly formed periodontal fibers was in no way different from fibers forming the periodontal membrane of the first and third molars. Eleven

* Abbott Laboratories.

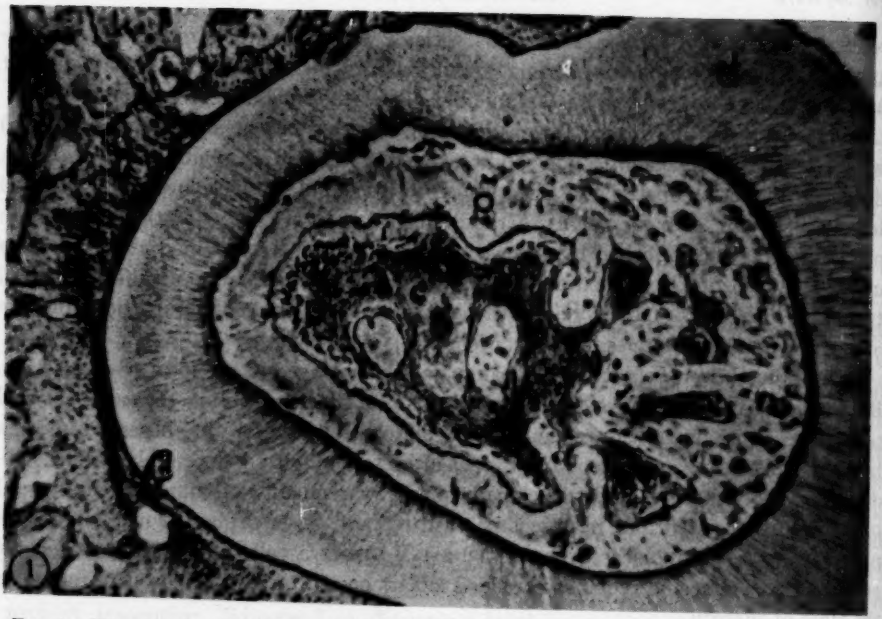

FIG. 1.—Transverse section of replanted molar tooth. *d*, dentin; *o*, osteodentin; *p*, *pulp*. (Orig. mag. ×84.)

FIG. 2.—Transverse section of replanted molar tooth. *d*, dentin; *o*, osteodentin; *b*, odontoblasts; *v*, vessel. (Orig. mag. ×366.)

teeth showed histologic evidence of root resorption. Acellular cementum was seen in the areas of cemental resorption. Some first and third molars exhibited root resorption which was as great as the resorption noted in three of the replants. No internal resorption was noted in any of the replants. Three of the replanted teeth exhibited pulps which were completely normal in histologic appearance, while six replants contained normal pulp tissue but included varying amounts of osteoid material. This osteoid material followed the dentino-pulpal junction (Figs. 1 and 2).

FIG. 3.—Longitudinal section, showing root of replanted tooth. *m*, periodontal membrane; *d*, dentin; *v*, vessel; *n*, regenerated nerve. Arrow points to layer of predentin. (Orig. mag. ×100.)

Regenerated nerves were observed in nine of the sixteen replants. Only those teeth which revealed normal histologic pulp or teeth containing some normal pulpal material, with the remaining pulpal area occupied by an osteoid material, exhibited regenerated nerve tissue. Normal pulpal tissue, including vessels, odontoblastic layer, and regenerated nerve tissue, could be seen in both root chambers and pulp canals (Figs. 3 and 4). In all teeth except one (Fig. 3), a distinct, dark-staining area was visible in the matured calcified dentin, which appeared to indicate reparative dentin (Fig. 5). The innermost layer of dentin, apparently laid down after the replantation procedure, had poorly organized tubules which were branched and fewer in number (Fig. 6). Root resorp-

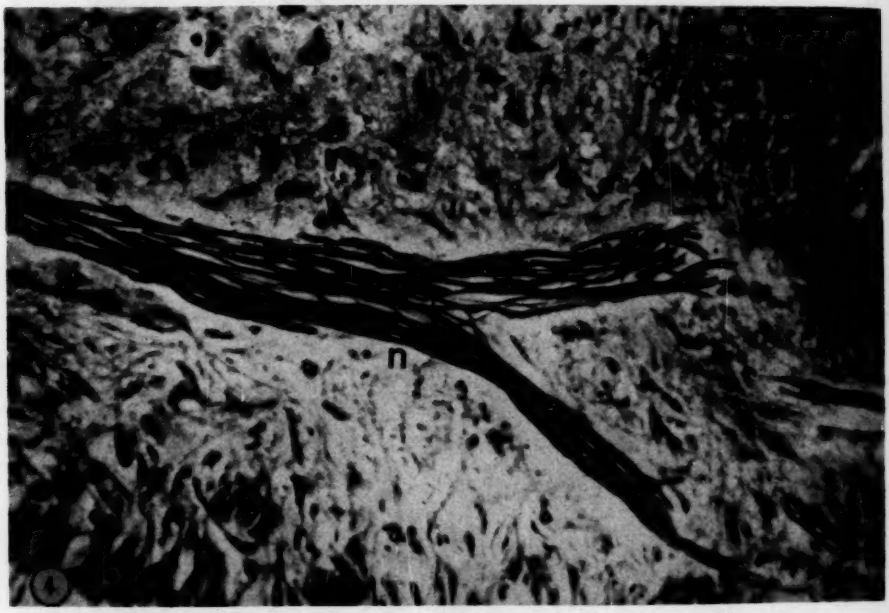

FIG. 4.—Section from pulp of replanted tooth. *n*, regenerated nerve; *b*, odontoblasts. (Orig. mag. ×440.)

tion was prominent in the replanted teeth. Nerve trunks appeared to traverse the root canals and approach the cuspal areas, where they branched into fibrils and filaments which terminated as beaded or free endings among the odontoblasts. Occasionally the filaments appeared to penetrate the predentin and dentin areas (Figs. 7 and 8). Terminal endings were most conspicuous in the cuspal areas and were not evident in sections of the root canal. Nerve tissue within the root canals seemed to end at or in the odontoblastic layer.

DISCUSSION

When nerves that are normal in their histologic appearance are demonstrated within the pulps of replanted teeth, it must be assumed that regeneration from the proximal stump through the root apex has occurred.

According to Guth,[14] after severance of a nerve, the stage of physical destruction of

myelin is followed by a second stage of chemical destruction. The axon undergoes deterioration and finally disappears entirely. The process of regeneration is one of axon outgrowth. Axon extensions with pseudopod-like structures travel along the neurilemma bands, which proliferate in a process wholly independent of axonal regeneration. The outgrowth of neurilemma cells and conversion to protoplasmic strands is of the greatest importance in regeneration, for it is along these proliferated mesial and distal

FIG. 5.—Longitudinal section of replanted tooth, showing dark-staining zone between dentin (*d*) and reparative dentin (*s*); *r*, root resorption. Pulp contains organized odontoblastic layer, vessels, and regenerated nerves; *a*, artifact. (Orig. mag. ×100.)

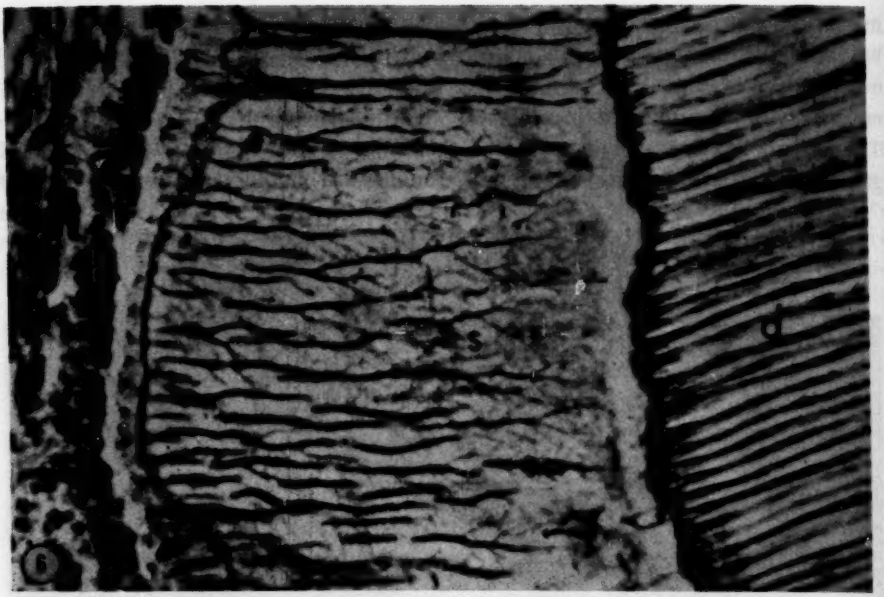

FIG. 6.—Section from replanted tooth. Dark-staining zone between dentin (*d*) and reparative dentin (*s*) indicates time of replantation procedure; *b*, odontoblasts. (Orig. mag. ×440.)

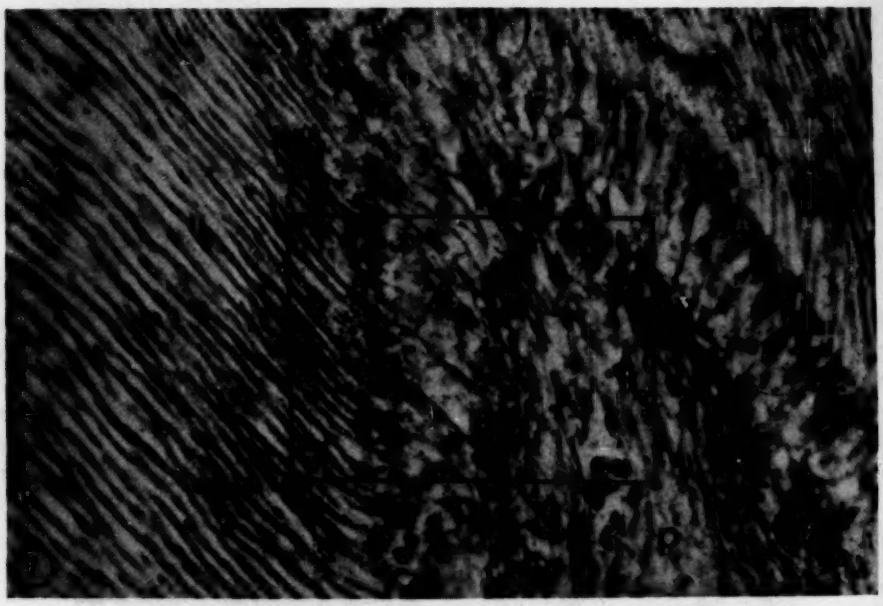

FIG. 7.—Longitudinal section of replanted tooth, showing nerve fibrils (arrows in area of odontoblasts *b*). *p*, pulp; *d*, dentin. (Orig. mag. ×440.)

cells that the axon travels.[15] Young[15] reported on the rate of advancement of the axon tip in the rabbit. After axon severance and a latent period of approximately 7.3 days, the axon advances 3–4 mm. per day. This rate of axon growth is slower at the periphery because of the greater distance of the developing axon from the cell body. In addition, growth rate decreases as the length of the regenerated nerve becomes greater because of an increased resistance of peripheral stump tissue or a decrease in outgrowth power.

While, in the study reported here, acrylic splints may have assisted in retention of the replants (all acrylic-splinted replants were maintained by the animals), it is doubtful that such means of tooth orientation aided any neurotrophic or contact guidance of

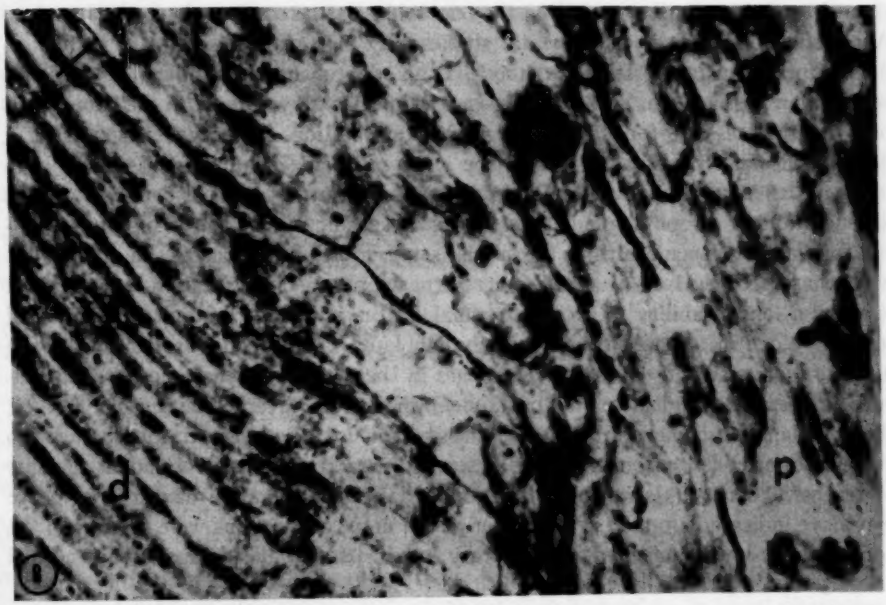

FIG 8.—Area of insert shown in Fig. 7. Regenerated nerve fibrils (*arrows*) can be seen passing from the pulp (*p*) into the dentin (*d*). (Orig. mag. ×970.)

the proximally proliferating axon to its peripheral termination. Half of the splinted replants showed evidence of vital pulp, while the other half of the splinted teeth showed necrotic pulpal tissue. Though not the focus of this study, it was noted that the amount of periodontal membrane retained on the molar replants at the time of extraction appeared to have no relationship to the quality of periodontal reattachment or amount of root resorption. Root resorption occurred in all replanted teeth except one.

Nerve regeneration occurred in all teeth which contained histologically normal pulp tissue. No nerve was observed in any of the necrotic pulps. Odontoblastic production of primary and reparative dentin and an osteoid material, perhaps more appropriately named "osteodentin," were observed in all replants containing regenerated nerve tissue. The osteodentin generally followed the pulpo-dentinal junction. In five of the successful replants, osteodentin was observed. When present, the osteodentin appeared to be deposited upon a layer of reparative dentin and to be produced by odontoblasts within

a pulp canal and chamber receiving few regenerated nerve fibers but exhibiting adequate pulpal revascularization.

Utilizing the facial nerve of the white rat, Weiss, Edds, and Cavanaugh[16] showed that mandibular nerve fibers unable to connect with their end organs remained small and did not acquire their full diameter, while uncapped regenerating fibers which reached their terminal connections achieved normal size. The end organ was shown to have the greatest importance on regenerated nerve. There was evidence that cells and fibers disconnected from their end organs might eventually undergo atrophy and degeneration. Speidel[17–19] demonstrated in the tadpole the close organizational unity between the sense organ and its specific nerve, while Avery and Rapp,[20] in an investigation of tooth innervation, suggested the odontoblast as a receptor of sensory impulses. Application of Speidel's experimental studies may help explain secondary and osteodentin formation in the replanted tooth if the odontoblast is considered as a special sense organ of the tooth. While the presence of specific nerve supply to the odontoblast may be unnecessary for its origin, growth, maturation, and regeneration, such a denervated organ could, according to the views of Speidel, be expected eventually to undergo structural deterioration. Deterioration may manifest itself as dedifferentiation, degeneration, or atrophy. Speidel stressed that a specific sensory nerve supply is essential for the long-range health of the special sense organ. In the case of an odontoblast which has been deprived of an adequate nerve supply, dedifferentiation may manifest itself as a loss of ability to produce normal dentin, with the resultant production of reparative dentin followed by osteodentin. Replants with osteodentin deposition may indicate regressive and degenerative changes in the peripheral sensory organs. In general, the replants studied in this experiment showed an inverse relationship between the abundance of demonstrable nerves and the amount of osteodentin production. No evidence of osteodentin formation was observed in any replants with abundant amounts of regenerated nerve tissue. A replant with a layer of reparative dentin in evidence may have indicated a period in which peripheral nerve connections were scarce and the odontoblastic layer underwent a temporary loss in its specialized capacity for normal primary dentin production.

This investigation has demonstrated the presence of nerve tissue in the canals and chambers of replanted Syrian hamster molars. Over 50 per cent of all maintained replants showed histologic evidence of peripheral nerve regeneration. Such nerve regeneration is of particular interest when coupled with the speculation that an interrelation exists between pulpal nerve and odontoblastic health and function. While this interrelationship appears to be worthy of consideration, it is recognized that other factors may affect odontoblastic activity in replanted teeth. The observation of regenerated nerve within replanted teeth broadens the opportunity for future investigations into the complexity of problems encountered in tooth replantation and transplantation procedures.

SUMMARY

The presence of regenerated nerve was demonstrated in nine out of sixteen replanted second molar teeth in the Syrian hamster.

An apparent correlation between the amount of nerve regeneration and the type of dentin production following tooth replantation was observed.

The quality of periodontal reattachment and root resorption was not dependent on the amount of vital periodontal membrane retained on the tooth root during replantation procedures.

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Permeability of Cavity Liners to Certain Agents

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The use of cavity liners and varnishes under silicate or zinc phosphate cement and other restorative materials is a routine procedure in many dental offices. Two of the common reasons cited for the use of these materials are (1) their ability to seal the dentinal tubules and preclude penetration of acid or other deleterious agents from the restorative materials and (2) as an aid in sealing cavity margins to prevent the ingress of saliva and debris between the restorative material and the cavity preparation. Thus it is apparent that the efficacy of these materials is believed to be primarily related to their ability to prevent penetration of certain substances.

A limited amount of research has been conducted in this area. P^{32}-labeled phosphoric acid and dyes have been employed to determine whether various lining materials would prevent penetration of phosphoric acid from dental cements into dentin.[1–5] However, generally the materials have been evaluated either on empirical clinical observations or on the basis of histologic studies comparing the incidence and severity of pulp damage in teeth in which the cavity was lined or unlined and filled with silicate cement.[1–13] For the most part, the results have been inconclusive. A recent report[13] has classified the varnish-type cavity liners as moderately effective protectives against acid, while another study has indicated that these agents are semipermeable membranes.[10] In view of the lack of adequate information concerning the permeability of these liners to certain agents, this research was initiated.

MATERIALS AND METHODS

Calcium solubility has long been used as a measure of the influence of certain chemicals on the solubility of tooth structure; therefore, it was employed in this investigation as the main criterion for measuring permeability of the cavity varnishes. For example, when the intact surface is treated with an aqueous solution of sodium fluoride, a reduction in enamel solubility is generally produced. Thus, if the cavity varnish is first applied and the same fluoride solution does not decrease enamel solubility, the fluoride solution has not penetrated the film.

Utilizing this principle, three commercial cavity liners* of the varnish type were

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* Copalite, H. J. Bosworth Co., Cavaseal, Kerr Mfg. Co., and Repelac, L. D. Caulk Co.

investigated. Since it is not the purpose of this particular investigation to compare specific brands but rather to indicate general characteristics of the materials, code letters will be used. Liners which are referred to as A and B are composed of natural gums and rosins, while C is a synthetic resin material.

Phosphoric acid, acetic acid, and sodium fluoride solutions were employed as the test media to measure the permeability of the liners. The phosphoric acid was included because it is an essential constituent of dental cements; the sodium fluoride solution, because fluorine is a component of most silicate cements; and acetic acid, because it is representative of organic acids present in the oral cavity. The permeability of the liners to the components of the restorative materials was also investigated.

Previous research has indicated that the fluoride present in silicate cement or when added to certain resin materials does reduce the acid solubility of adjoining enamel or dentin.[14–17] Thus some of these materials were included, in order to determine whether the lining agents would act as a barrier to the fluoride present in those materials. Two silicate cements and two resins were used in conjunction with the liners, the calcium solubility of enamel being measured before and after contact with the restorative materials. According to the respective manufacturers, the first silicate* is prepared with a fluoride flux, while the second† contains no fluoride, as a beryllium flux is used. One of the resins‡ contains an unknown concentration of both sodium and stannous fluoride, while the other resin§ was an experimental one with 2 per cent sodium fluoride added to the resin powder.

All experiments were conducted on the intact enamel surfaces of freshly extracted teeth. Teeth were never permitted to become desiccated during the course of the tests. A window-type solubility test that had been used previously in studies related to the effects of restorative materials on the solubility of tooth tissue was employed.[15, 16] Very briefly, the procedure consisted of thoroughly cleaning and polishing sound central incisor teeth. The roots were separated from the crowns, and nichrome wires were inserted in the pulp chambers. The crowns were completely sealed with a combination of wax and electrical tape except for a circle of enamel 3.5 mm. in diameter left exposed on the labial surface.

Each crown was suspended by means of the wire in 10 ml. of an acid decalcifying solution and agitated for the prescribed period. A 30-minute decalcifying time was used with acetic acid and a 15-minute period for phosphoric acid. The amount of calcium dissolved from the untreated exposed area of enamel during these time intervals was measured, and those data served as the control for the particular tooth. The teeth were then resurfaced by buffing on a felt wheel with a slurry of levigated alumina. When all traces of the acid etch had been removed, the experimental treatment was carried out. The windows were then replaced, and the decalcification test repeated. Thus the effects of the experimental treatment on the solubility of the teeth were demonstrated.

Preliminary tests were conducted in order to ascertain whether the cavity varnishes themselves or the organic solvents used for removing them from the tooth surface ap-

* Filling Porcelain, S. S. White Dental Mfg. Co.

† Berylite, Ames Co., Inc.

‡ FluorOn, American Consolidated Mfg. Co.

§ Experimental resin supplied through courtesy of L. D. Caulk Co.

preciably affected the rate of calcium dissolution. After determining the original solubility of the surfaces, coats of the varnishes were applied, and the teeth were placed in a humidor. Twenty-four hours later the varnishes were removed by the appropriate solvents, and solubility was again measured. Neither the liners nor the solvents had any apparent effect on enamel solubility.

In order to investigate the permeability of the various varnishes to the acids, generous coatings of the materials were applied to the tooth surfaces. The teeth were placed in a humidor until the varnishes dried. Windows were then placed over the coatings, care being taken not to disturb the continuity of the films. These coated windows were then subjected to the acid. The amount of calcium dissolved during 15-minute and 24-hour immersion in phosphoric acid and 30-minute and 24-hour immersion in acetic acid was used as a means of detecting the degree to which the respective acids were able to permeate the coatings. The initial 15- and 30-minute immersion times were established on the basis of preliminary tests conducted to determine the time intervals at which optimum quantities of calcium were leached from the enamel surfaces in each decalcifying medium.

To determine whether the varnishes would permit penetration of fluorine, varnish-protected teeth were subjected to solutions of 2 per cent sodium fluoride for periods of 16 minutes and 24 hours. The varnishes were then removed, the solubility in acetic acid measured, and these changes compared with those induced in enamel by application of the fluoride solution directly to the tooth surface.

The effects of the restorative materials, when used with the varnishes, were studied by first applying the varnishes to the teeth, allowing them to dry, and then applying the restorative materials to the varnish-protected surfaces. The teeth were stored in distilled water at 37° C. The water was changed daily, and at the end of the 2 weeks the materials were removed and enamel solubility determined.

Both the phosphoric and the acetic acid solutions were 0.2 M concentration with the pH adjusted to 2.5. Acetic acid was used for all decalcification except, of course, in those instances where permeability to phosphoric acid was specifically being tested. The method of calcium analysis was that of Solomon, Gabrio, and Smith.[18]

Over 600 teeth were used during the course of this investigation. Each series of tests was conducted on a minimum of 8 teeth, while as many as 60 were used in some instances. The data shown are the average results obtained with each experimental group.

RESULTS

The average quantities of calcium dissolved by the two acids from the varnish-protected enamel, as compared with that dissolved from unprotected enamel, are shown in Table 1. These results indicate that, although none of these cavity liners acted as a complete barrier to either acetic or phosphoric acid, they did afford considerable protection to the tooth against the attack of these acids. During 30 minutes' immersion in acetic acid, approximately 80–90 per cent more calcium was leached from unprotected enamel surfaces than from those protected by the varnishes. Comparable results were obtained with phosphoric acid. Even though in most instances the calcium dissolved from the protected surfaces did increase during the 24-hour exposure to the acids, the calcium solubility figures did not approach those of the controls.

The results on the effectiveness of topically applied sodium fluoride, as influenced by the cavity varnishes, are shown in Figure 1. The mean reduction in enamel solubility was 10.0 per cent when the unprotected surfaces were treated for 16 minutes with 2 per cent sodium fluoride. There was no significant change in the solubility of the varnish-coated enamel, indicating that sodium fluoride in appreciable quantity had not penetrated the films during that time. However, when the varnish-protected teeth were

TABLE 1

QUANTITY OF CALCIUM DISSOLVED BY ACETIC ACID AND PHOSPHORIC ACID FROM ENAMEL SURFACES PROTECTED BY VARNISHES AS COMPARED WITH THAT DISSOLVED FROM UNPROTECTED SURFACES (CONTROL)

LINER	ACID	CONTROL (Mg. % Ca) (30 Min.)	EXPERIMENTAL	
			Mg. % Ca (30 Min.)	Mg. % Ca (24 Hr.)
A	HAc	1.425	0.193	0.168
B	HAc	1.888	0.167	0.186
C	HAc	1.270	0.146	0.310
		(15 min.)	(15 Min.)	(24 Hr.)
A	H_3PO_4	1.231	0.156	0.224
B	H_3PO_4	1.085	0.137	0.227
C	H_3PO_4	1.270	0.261	0.328

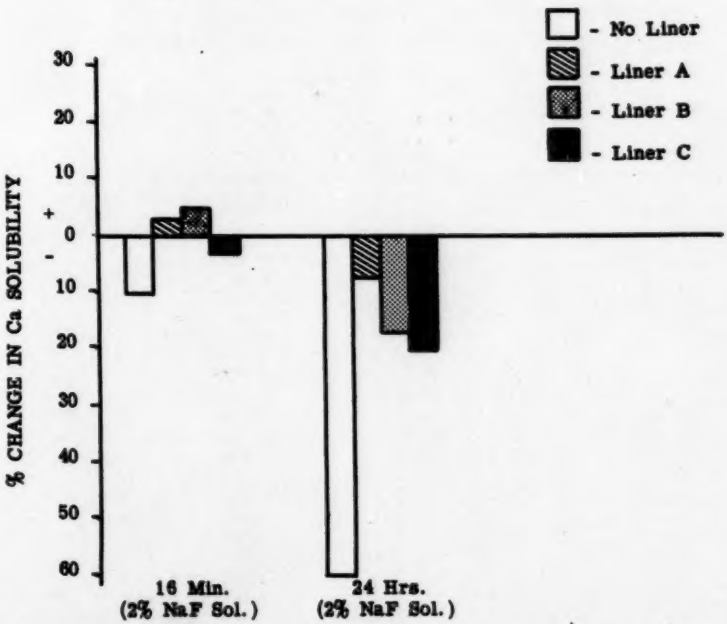

FIG. 1.—Change in calcium solubility of intact enamel as induced by topical application of 2 per cent sodium fluoride solution to surfaces coated with various varnishes.

immersed in sodium fluoride solution for 24 hours, there was evidence that the fluoride had penetrated the liners. Although the decreases in enamel solubility were markedly less than when the solution was applied directly to the enamel, definite reductions were secured with materials B and C. The average reduction obtained with the use of liner A was less; however, it should be pointed out that a much greater deviation was noted in the individual data for this liner. Nineteen of the 45 teeth tested produced reductions in solubility exceeding 10 per cent, while the remainder demonstrated little change or even increases in solubility. In order to illustrate this variation, a sample of the individual data appears in Table 2. The standard deviation for this group of specimens was ±13.5 per cent, or approximately twice as great as that demonstrated by the other test groups. The standard deviations generally approached ±6.5 per cent.

In Figure 2 the effects of the various restorative materials on enamel solubility, with and without the use of the liners, can be seen. The results indicate that the effect on the enamel solubility of the silicate which contained fluorine was generally decreased when the liners were used. Even though considerable variation was again observed in individual results, there was an appreciable decrease in the solubility of the tooth structure when the silicate was applied over liners A and B. The averages indicate less change in the teeth coated with brand C; however, a number of the teeth did exhibit rather marked reductions.

TABLE 2

SAMPLE OF INDIVIDUAL DATA OBTAINED WITH APPLICATION OF 2 PER CENT SODIUM FLUORIDE TO SURFACES COATED WITH LINER A TO ILLUSTRATE THE GREAT VARIATION IN SPECIMENS FOUND WITH THIS PARTICULAR MATERIAL

Tooth No.	Liner A (24-Hr. Immersion in 2 Per Cent NaF)			
	Control (Mg. % Ca)	Experimental (Mg. % Ca)	Mg. % Difference	% Difference
1	1.540	1.412	−0.128	− 8.3
2	1.725	1.833	+0.108	+ 6.3
3	1.754	1.567	−0.187	−10.7
4	1.611	1.395	−0.216	−13.4
5	1.104	1.126	+0.022	+ 2.0
6	1.205	1.352	+0.147	+12.2
7	1.443	1.267	−0.166	−11.5
8	1.710	1.402	−0.308	−18.0
9	1.216	1.242	+0.026	+ 2.1
10	1.693	1.275	−0.418	−24.7
11	1.477	1.363	−0.114	− 7.7
12	1.103	1.258	+0.155	+14.4
13	1.272	1.356	+0.084	+ 6.6
14	1.446	1.109	−0.337	−23.3
15	1.366	1.298	−0.068	− 5.0
16	1.836	1.319	−0.517	−28.2
17	1.623	1.058	−0.565	−34.8
18	1.387	1.314	−0.073	− 5.3
19	1.057	1.122	+0.065	+ 6.2
20	1.468	1.277	−0.191	−13.0
21	1.118	1.220	+0.102	+ 9.1
Average				(− 6.9)

Previous research with the silicate which contained *no* fluoride has shown that contact with this material definitely increased the solubility of intact enamel surfaces.[15] It was interesting to note that the liners generally reduced the effect. There was no change in solubility when liner C was applied to the surface. The fluoride-free silicate exerted only minimal effects on enamel coated with the other two liners.

None of the liners showed any marked influence on solubility when used in conjunction with the two fluoride resins. Apparently, the fluoride present in the materials readily penetrated the varnishes. However, these results are possibly not surprising, since all the liners were softened by methyl methacrylate monomer.

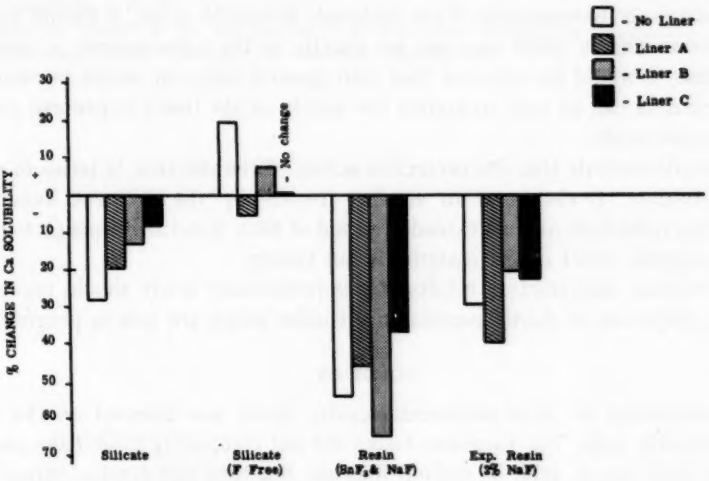

FIG. 2.—Change in calcium solubility of intact enamel as induced by restorative materials when applied to unprotected and varnish-protected surfaces.

DISCUSSION

It should be pointed out that the relatively high deviation observed in the behavior of individual specimens is typical of this particular type of test. Although this must be recognized as a shortcoming of the method, it is felt that the advantages of employing the intact surface to predict clinical effects of materials far outweigh the advantages of obtaining somewhat more precise results through use of powdered tooth structure. In fact, the differences displayed by individual teeth may well reflect differences in the susceptibility of specific teeth to a particular treatment.

In this present study some of the variation encountered was undoubtedly due to the difficulty of obtaining a continuous coating, of uniform thickness, with the cavity liners.

The problem was undoubtedly magnified through use of the highly polished tooth surface and the large area which had to be covered. Other factors may well be involved, but, regardless of the causes, it is felt that this work does indicate the difficulty of controlling all the variables involved in the use of these materials and hence tends to support some of the controversial results obtained by other investigators in this area.

The trends indicated were quite definite, and the results of the experiment corrobo-

rate the observations of Massler,[13] Zander,[7, 10] and others[3, 5] in that cavity varnishes do not entirely prevent the penetration of acids but that they do afford some measure of protection to the tooth against phosphoric acid. The amount of protection provided would seem to be dependent on the length of time that free phosphoric acid remains in the cement or the pH of the acid at the time when it finally penetrates the liner. Since these facts have never been completely established and are without doubt partially dependent on manipulation the degree of protection afforded by the liner still remains controversial. However, the observation that no significant increase in enamel solubility resulted from use of a fluoride-free silicate when the various liners were applied would lend further support to the role of these materials as protective agents.

In regard to the permeability of the materials to organic acids, it should be pointed out that other organic acids may not act exactly in the same manner as acetic acid. Nevertheless, it would be expected that their general behavior would be similar, and hence these data can be used to predict the ability of the liners to prevent passage of certain organic acids.

The data do indicate that the protective action of the silicates, in terms of reducing enamel solubility, is decreased to varying degrees by the different lining agents. Whether this reduction, over an extended period of time, is actually enough to influence the anticariogenic effect of the material is not known.

The occasional contradictory results of this preliminary study should become clarified upon completion of dentin permeability studies which are now in progress.

SUMMARY

The permeability of three commercial cavity liners was assessed on the basis of enamel solubility tests. The varnishes tested did not completely inhibit the passage of phosphoric acid, acetic acid, or sodium fluoride, but they did greatly reduce it. The effects which some restorative materials had on tooth tissue were modified when placed over a varnish. The tendency for a fluoride-free silicate to increase enamel solubility was generally negated, while the protective effects of a fluoride-containing silicate were reduced. The action of the resins which contained fluoride was not altered by the varnishes.

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Temporary Uterine Circulatory Arrest as a Cause of Abnormal Fetal Development

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A great number and variety of experimentally induced factors have been implicated in the genesis of congenital abnormalities.[1] This seemingly endless search for newer teratogenic agents must, nevertheless, continue if we are to understand better the patterns of progression and transformation involved in deviations from normal development.

Prior to birth, the fetus is dependent on the placenta and the umbilical cord for its oxygen supply, and any interference with the function of either could be followed by hypoxia. Thus such untoward events as premature separation of the placenta from the uterine wall and twisting or knotting of the umbilical cord can induce a condition of fetal hypoxia. Although these disturbances of pregnancy in humans relate primarily to the period approaching parturition and are important considerations in current searching for causes of such abnormalities of the newborn as cerebral palsy,[2] some attention has been directed in experimental studies to the consequences of hypoxia occurring during earlier stages of gestation.[3–7] While these reports have been few in number, their methods of procedure have been quite varied. Thus, in matters of strain and species differences, duration of reduced atmospheric pressure, rapidity of compression and decompression, and stage of pregnancy when pressures were altered, little basis exists for comparative analysis of findings.

Ingalls, Curley, and Prindle[3] subjected mice at varying stages of pregnancy to reduced atmospheric pressures of from 260 to 280 mm. Hg for a period of 5 hours. Animals studied were albinos selected at random from a closed colony maintained at Harvard University for the previous 5 years. Based on observations in 340 experimental and 138 control animals, it was concluded that maternal hypoxia is a significant cause of congenital deformity. Of the total number of 1,882 experimental and 880 control fetuses examined, 127 showed gross defects involving primarily the skeletal tissues. Palatal clefts were found in 13 instances, 9 of which occurred in mothers exposed during the fifteenth day of gestation. No defects were found in the offspring of control mothers.

Further demonstrating the relationship of hypoxia to congenital defects in mice, Murakami, Kameyama, and Kato[4] reported a reduction in litter size and number, as well as defects predominantly of the central nervous system. In contrast to the studies

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of Ingalls *et al.*,[3] Murakami *et al.*[4] were concerned only with the early period of gestation.

In establishing whether differences in animal strain and animal species can influence the expression of induced anomalies, several studies may be cited. Ingalls, Avis, Curley, and Temin[5] compared the reactions to hypoxia of five inbred strains of mice of known genetic constitution. Limiting their study to high-altitude exposure on the ninth and fifteenth gestational days, various congenital defects were noted which were clearly influenced by strain constitution. Thus, of 18 palatal clefts observed (all associated with harelip), 17 were limited to a single strain. In another study by Werthemann and Reiniger, it was noted that a reduction in litter size, as well as extensive eye deformities, occurred in the young of white rats exposed to reduced atmospheric pressure of from 300 to 400 mm. Hg for a period of 48 hours during the first 8 days of gestation.[6] Somewhat different findings were reported in the case of rabbits. Degenhardt and Kladetzky[7] exposed this species to a pressure of 226 mm. Hg for a period of 4–7 hours between the seventh and fifteenth days of gestation and noted developmental disorders, predominantly of the axial skeleton, only after exposure on the eighth, ninth, or tenth day of pregnancy.

A more recent study by Curley and Ingalls[8] was designed to evaluate the effect of hypoxia without the complicating variables of compression and decompression. Subjecting mice, at normal atmospheric pressure, to an oxygen concentration equivalent to that at 226 mm. Hg, they observed malformations which were similar to those previously reported after high-altitude-chamber exposure.

The present study was designed primarily to evaluate the effect on palatal development of a well-controlled, experimental procedure for inducing fetal hypoxia, namely, a temporary arrest of maternal blood supply to the placenta.

EXPERIMENTAL METHODS

By means of daily vaginal smears for demonstration of spermatozoa, the onset of gestation was established in 249 Sprague-Dawley rats. The day following the appearance of a positive smear was recorded as the first day of pregnancy.

At periods of gestation ranging from 7 to 17 days, the circulation to the right or left uterine horn was arrested by clamping the uterine and uteric arteries for periods of 10–60 minutes. The latter range was established on the basis of preliminary findings which showed that 60 minutes of circulatory arrest produced a severe anoxia without causing invariable resorption of all fetuses. In similar fashion, preliminary observations on the rat showed that the thirteenth through the fifteenth gestational days are the critical periods of palatal development and closure. Thus greatest attention was given to these stages of embryogenesis, although earlier days of gestation also were studied, in order to determine whether hypoxia might exert a long-term effect on palatal development. In each instance the uterine horn with the largest number of implantation sites was selected as the experimental side. The vascular supply to the opposite uterine horn was not occluded and thereby provided a source of control fetuses in each experimental animal. Although some question of bias may be related to the procedure of arbitrarily selecting experimental and control sides, the constancy of the bias for each gestational day and duration of circulatory arrest and the procedure of direct comparison between experimental and control horns make it quite negligible.

All operative procedures were done under sodium nembutal anesthesia, supplemented, as necessary, with ether. Both uterine horns were carefully exposed through a mid-line abdominal incision, in order to identify the adnexa and count all fetuses. Circulatory arrest was accomplished by means of small bulldog clamps, modified in design to provide an optimum degree of pressure on vessel walls without undue trauma. Except for the procedure of clamping, which was completed within a period of 15–30 seconds, both uterine horns received a comparable degree of handling. Assurance of successful circulatory arrest was provided by the rapid onset of vascular engorgement on the treated side. Immediately following this procedure, both uterine horns were returned to the body cavity and the incision closed with quick-tie sutures. After the scheduled period of circulatory arrest, the uterine horns were again exposed, the clamps removed, and the uterus returned to its normal position for final closure of the wound.

One day prior to expected day of spontaneous term delivery, Caesarean sections were performed, and the extracted young were examined for defects. Sites of fetal resorption also were noted.

RESULTS

Table 1 categorizes the 249 gravid rats of this study according to experimental procedure (day of gestation and duration of circulatory arrest) and related findings in fetal development. The descriptive category of "mothers with affected young" relates to those that contain deformed, resorbed, or macerated products of conception. Two deformities occurred in the control fetuses. One of these was a cleft palate and the other a cleft in combination with a limb deformity. Both were found in litters of mothers that had been treated on the fourteenth day of gestation. In contrast, the experimental series showed 26 young with deformities. There were 13 instances of palatal clefts, 8 of which occurred in mothers that had been treated on the fourteenth gestational day (Table 2). Of the 16 young with limb defects, 5 also had cleft palates. The extremity defects included varying degrees of agenesis, shortening, malrotation, and syndactyly. One exencephaly and another generally misshapen fetus were observed in litters that had been subjected to anoxia on the twelfth and thirteenth gestational days, respectively.

From the design of the experiment, it was possible to study not only the effect of vascular occlusion on palatal development but also its relationship to fetal death, i.e., incidence of maceration and resorption. Shown in Table 3 are the proportions of macerated-resorbed young as related to gestational days of circulatory arrest and durations of arrest. It may be noted that the summarized average incidence of fetal deaths in the experimental horns shows a striking increase as the duration of circulatory arrest is extended, whereas the average in the control horns remains low and approximately the same for all periods of vascular occlusion. With reference to gestational period, it is apparent that the thirteenth day is the most critical for fetuses in the experimental horns, the summarized totals showing a sharp rise from 0.09 on the seventh day to 0.55 on the thirteenth day, and then a moderate decline to 0.34 on the seventeenth day. The summarized mortality incidence for control fetuses follows the same general pattern but remains at a markedly lower level. The differences between control and experimental fetus mortality that exist at certain periods of experimental procedure (day of gestation or duration of circulatory arrest) and not at others dem-

TABLE 1

EFFECT OF UTERINE CIRCULATORY ARREST ON FETAL DEVELOPMENT

GESTATIONAL DAY OF CIRCULATORY ARREST	DURATION OF CIRCULATORY ARREST (MINUTES)	NO. OF TREATED MOTHERS	EXPERIMENTAL HORN					CONTROL HORN				
			Total No. of Fetuses	No. of Normal Fetuses	No. of Deformed Fetuses	No. of Macerated or Resorbed Fetuses	Mothers with Affected Young	Total No. of Fetuses	No. of Normal Fetuses	No. of Deformed Fetuses	No. of Macerated or Resorbed Fetuses	Mothers with Affected Young
7–8	10	6	34	31	.....	3	3	24	23	.....	1	1
	15	6	33	32	.....	1	1	23	21	.....	2	2
	30	8	46	39	.....	7	4	21	21	.....	0	0
	45	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	60	7	46	41	.....	5	4	30	30	.....	0	0
Total	.....	27	159	143	.....	16	12	98	95	.....	3	3
10	10	5	28	27	.....	1	1	21	19	.....	2	2
	15	5	25	23	.....	2	1	16	15	.....	1	1
	30	5	28	24	.....	4	4	22	20	.....	2	1
	45	5	33	29	.....	4	3	19	18	.....	1	1
	60	5	32	15	.....	17	5	18	17	.....	1	1
Total	.....	25	146	118	.....	28	14	96	89	.....	7	6
12	10	5	29	23	.....	6	3	13	11	.....	2	1
	15	5	31	19	1	11	5	19	19	.....	0	0
	30	5	29	19	.....	10	3	18	15	.....	3	3
	45	5	26	10	1	15	5	21	20	.....	1	1
	60	10	55	24	.....	31	10	34	29	.....	5	3
Total	.....	30	170	95	2	73	26	105	94	.....	11	8
13	10	6	35	20	1	14	5	26	22	.....	4	4
	15	6	40	22	2	16	5	18	16	.....	2	2
	30	7	37	18	.....	19	7	31	26	.....	5	2
	45	10	53	14	1	38	10	35	32	.....	3	3
	60	8	53	9	.....	44	8	21	18	.....	3	2
Total	.....	37	218	83	4	131	35	131	114	.....	17	13
14	10	5	28	23	.....	5	4	19	19	.....	0	0
	15	13	67	45	1	21	10	41	34	1	6	4
	30	11	63	30	3	30	11	33	29	1	3	2
	45	7	38	14	1	23	6	23	18	.....	5	5
	60	25	150	54	8	88	22	91	84	.....	7	7
Total	.....	61	346	166	13	167	53	207	184	2	21	18
15	10	6	34	32	.....	2	2	22	19	.....	3	2
	15	6	35	31	.....	4	3	19	18	.....	1	1
	30	9	49	40	.....	9	5	30	28	.....	2	2
	45	10	62	22	6	34	9	37	36	.....	1	1
	60	10	55	12	1	42	10	29	28	.....	1	1
Total	.....	41	235	137	7	91	29	137	129	.....	8	7
17	10	5	23	21	.....	2	1	18	17	.....	1	1
	15	5	31	27	.....	4	4	14	11	.....	3	3
	30	6	37	25	.....	12	5	15	14	.....	1	1
	45	6	39	20	.....	19	6	16	16	.....	0	0
	60	6	32	10	.....	22	5	17	16	.....	1	1
Total	.....	28	162	103	.....	59	21	80	74	.....	6	6
Inclusive gestational period of study (7–17 days)	10	38	211	177	1	33	19	143	130	.....	13	11
	15	46	262	199	4	59	29	150	134	1	15	12
	30	51	289	195	3	91	39	170	153	1	16	11
	45	43	251	109	9	133	39	151	140	.....	11	11
	60	71	423	165	9	249	64	240	222	.....	18	15
Total	.....	249	1,436	845	26	565	190	854	779	2	73	60

TABLE 2

Occurrence of Deformities in Experimental Young

Gestational Day of Circulatory Arrest	Duration of Circulatory Arrest (Minutes)	No. of Viable Young Examined	Type of Deformity				Total Deformities	Per Cent of Deformed Young
			Cleft Palate	Cleft Palate + Limb Defects	Limb Defects	Other Malformations		
12	15	21	.....	.....	.....	1	1	4.8
	45	12	.....	.....	1	.....	1	8.3
13	10	22	1	.....	.....	.....	1	4.5
	15	26	1	.....	.....	1	2	7.7
	45	16	.....	.....	1	.....	1	6.3
14	15	47	1	.....	.....	.....	1	2.1
	30	36	1	.....	2	.....	3	8.3
	45	16	1	.....	.....	.....	1	6.3
	60	70	2	3	3	.....	8	11.4
15	45	34	.....	2	4	.....	6	17.6
	60	14	1	.....	.....	.....	1	7.1
Total	.....	314	8	5	11	2	26	.....

TABLE 3

Proportion of Macerated and Resorbed Fetuses

Gestational Day of Circulatory Arrest	Duration of Circulatory Arrest										Summarized Average Proportion of Fetal Deaths	
	10 Min.		15 Min.		30 Min.		45 Min.		60 Min.			
	Exper.	Cont.	Exper.	Cont.	Exper.	Cont.	Exper.	Cont.	Exper.	Cont.	Exper.	Cont.
7–8	0.09	0.04	0.03	0.09	0.15	0.00	.....	.....	0.11	0.00	0.09	0.03
10	0.04	0.10	0.08	0.06	0.14	0.09	0.12	0.05	0.53	0.06	0.19	0.07
12	0.21	0.15	0.35	0.00	0.34	0.17	0.58	0.05	0.56	0.15	0.43	0.11
13	0.40	0.15	0.40	0.11	0.51	0.16	0.72	0.09	0.83	0.14	0.55	0.14
14	0.18	0.00	0.31	0.15	0.48	0.09	0.61	0.22	0.59	0.08	0.45	0.11
15	0.06	0.14	0.11	0.05	0.18	0.07	0.55	0.03	0.76	0.03	0 37	0.08
17	0.09	0.06	0.13	0.21	0.32	0.07	0.49	0.00	0.69	0.06	0.34	0.09
Sum. av. prop. of fetal deaths	0.16	0.09	0.21	0.12	0.27	0.11	0.51	0.09	0.59	0.07	.....	.....

onstrate the existing interactions. A similar correlation and interaction may be shown by an analysis of the number of mothers with affected young.

By an analysis of variance, pronounced differences were found in the proportions of fetal deaths between the experimental and control horns ($P < 0.01$). Statistically significant differences were also found in the proportions of fetal deaths between gestational days, irrespective of duration of circulatory arrest, and between periods of circulatory arrest, regardless of gestational day.

DISCUSSION

Although induction of hypoxia in pregnant animals by methods of lowered concentration of oxygen molecules in air mixtures or by reduced atmospheric pressure has been shown to cause congenital malformations and fetal death, the nature of the insult to the parent animal may be so generalized as to mediate its damaging effects on fetal development through diverse channels of metabolic influence. Other more indirect procedures that have been used for inducing hypoxia in pregnant animals have shown rather contradictory results. For example, Wilson[9] found little or no untoward effect on the offspring of rats subjected to episodes of hemorrhagic anemia during varying stages of pregnancy. In another experimental approach, Nishimura and Nakai[10] administered nicotine to pregnant mice, with resultant depressive action on the medullary respiratory centers, which in turn caused a variety of malformations, predominantly of the skeletal system. However, when a similar study was undertaken with rats, the offspring were unaffected.[11]

A principal objective of the present study was to devise a technique for circulatory interference that might be better controlled and more direct in its influence on fetal tissues than previously reported studies and also be somewhat analagous to practical clinical considerations of abnormal function of the placenta or umbilical cord which could lead to deficient maternal-fetal transport of respiratory gases and nutriments. It is of interest to note that, despite such differences in experimental procedure as well as in animal species, the crude rate of palatal clefts in our study (9.1 per thousand experimental fetuses) is not significantly at variance with that found by others (6.9 per thousand reported by Ingalls, Curley, and Prindle[4]). In accordance with the belief that a frequent cause of intrauterine death is interference with normal oxygenation,[12] Altland[13] found a 24.3 per cent fetal death rate in white rats exposed to 18,000 feet altitude for periods of 4 hours daily throughout the course of pregnancy. In contrast, his control group showed a 4.2 per cent incidence of resorptions. A somewhat higher percentage of fetal deaths was observed in the present study, namely, 39.9 per cent in experimentals and 8.5 per cent in controls.

Since gestation in the rat and mouse proceeds at such a rapid rate, the accuracy of timing of tissue and organ system development becomes a most critical factor in teratologic studies. In evaluating hypoxia as an etiologic factor in palatal clefts, it is important to consider the time of normally occurring palatal closure. Thus the palatine shelves of the Sprague-Dawley rat, as observed in this study, complete their mid-line fusion during the early part of the sixteenth gestational day, whereas various strains of mice are reported to reach the same stage of development at periods ranging from the early fourteenth through the fifteenth day of embryogenesis.[8, 14] However, much of the latter discrepancy in recorded time of palatal closure could well be related to

different methods of timing the onset of gestation, Walker and Fraser[14] recording the day of vaginal plug appearance as zero and Ingalls *et al.*[3] counting it as day 1.

While it is a highly questionable practice to make comparisons of experimental animal observations with findings in man, it is of some interest to note the study of Otis and Brent[15] which sought to establish the equivalent ages of mouse and human embryos at progressive periods of development. Based on their findings, it is perhaps significant that the 14½–15½-day stage in the mouse corresponds to the 7–10-week period of development in the human, these being the approximate times of normal palatal closure in the respective species.

Since the occurrence and nature of congenital anomalies are related to such variable and complicating factors as the type of injurious agent present and the time of embryogenesis during which the insult is applied, no simple cause-and-effect relationship can be arbitrarily inferred from the findings in the present study. Presumably, in addition to the specificity or non-specificity of agents for eliciting certain teratologic changes,[16–18] each organ or tissue has a critical time of development during which it is most vulnerable to any given agent. Thus any interruption of the developmental process may cause injury to those tissues or parts that are undergoing rapid proliferation and budding and cause little or no ill effects to the rest of the embryo.[19] For an abnormality such as cleft palate to occur, an interruption of development must necessarily occur before or during palatal closure. From the results of this study it can be inferred that circulatory arrest, with consequent deprivation of oxygen, is detrimental to palatal development as early as 2 days, and occasionally 3 days, prior to expected time of closure. Although it is a moot question whether this is the period of greatest tissue activity in the histogenesis of palatal development, the hypoxic insult is obviously of such a nature as to prevent palatal closure by the avenue either of direct damage to tissues or of retardation of growth potential.

SUMMARY

An attempt was made to evaluate the effect on fetal development of a temporary hypoxia produced by an arrest of maternal blood supply to the placenta. By means of small bulldog clamps, circulation to one uterine horn was occluded in 249 gravid Sprague-Dawley rats for periods of 10–60 minutes at stages of pregnancy ranging from 7 to 17 days. The opposite horn served as a source of control fetuses. Principal findings were as follows:

Of the 871 viable experimental young that were examined on the twentieth gestational day, 26 were grossly abnormal. Thirteen of these had cleft palates, and 16 showed limb defects. One exencephaly and a generally misshapen fetus also were observed.

There were 565 macerated or resorbed experimental fetuses, the highest incidence of fetal death occurring after circulatory arrest on the thirteenth gestational day.

In the control series, there were 779 viable normal fetuses, 2 deformed young, and 73 that were macerated or resorbed.

The findings indicate that palatal development is affected by interference of circulation to the placenta, particularly when occlusion occurs on the fourteenth or fifteenth day of gestation. Similarly, a statistically significant incidence of fetal death at the

longer durations of arrest is related to circulatory interference, the greatest incidence (83 per cent) being after 1 hour of occlusion on the thirteenth day.

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Dental Caries in Albino Rats Fed Diets Containing Different Amounts of Calcium and Phosphorus with the Same Ca/P Ratio

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In earlier experiments it was found that the cariogenicity of a high-sucrose diet was reduced when the Ca/P ratio of the diet was changed by the addition of phosphate to the diet without altering the calcium content.[1] Later it was shown that the diet could likewise be made less cariogenic when the Ca/P ratio was changed by adding calcium to the diet and keeping the phosphorus at a constant level.[2] In the first experiment a progressive decrease in the number of carious lesions and the caries score occurred up to a certain point with a progressive decrease in the Ca/P ratio, whereas in the second experiment this relationship was reversed: a decrease in caries conduciveness of the diet was associated with an increase in the Ca/P ratio. These findings were taken to indicate that the changes induced in the cariogenic properties of our high-sucrose diet, by altering the calcium and phosphorus content, were due to differences in the actual calcium and phosphorus content of the diets and not to differences in their Ca/P ratios. The evidence for this deduction, however, was admittedly indirect, inasmuch as in each experiment the Ca/P ratio was altered when either calcium or phosphorus was added to the diet. In the present study our conclusion drawn from our previous experiments was submitted to a direct test by increasing the calcium and phosphorus of the diet without changing the Ca/P ratio.

EXPERIMENTAL METHODS

Sixty albino rats of the Emory-Wistar strain were selected at weaning in groups of four from the same litter and were sialoadenectomized. The fifteen quadruplicate groups from time of weaning were fed four diets that differed from each other only in their calcium and phosphorus content. The basic high-sucrose diet employed in these experiments is described elsewhere.[3] Diet No. 1 contained 0.25 per cent calcium and the same amount of phosphorus. The other diets contained, respectively, 2, 4, and 6 times as much calcium and phosphorus as did diet No. 1. The Ca/P ratio was therefore the same in all four diets. The calcium and phosphorus were increased by adding calculated amounts of $CaCO_3$ and H_3PO_4 in the preparation of the salt mixture, which has been described elsewhere.[4] Whenever necessary, adjustments were made until the desired calcium and phosphorus content could be verified by analysis. After a feeding period

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of 80 days on the experimental diets, the animals were sacrificed, and the teeth were examined and scored for caries by our usual procedure.[3]

RESULTS

Increasing the calcium and phosphorus content of the diet from 0.25 to 0.50 and 1.00 per cent resulted in a progressive decrease in the number of carious lesions and in the caries score, which, as shown in Table 1, was statistically significant. Further increase of the calcium and phosphorus to 1.5 per cent did not produce an additional decrease in the caries-conduciveness of the diet.

TABLE 1

DENTAL CARIES IN ALBINO RATS FED SAME HIGH-SUGAR DIET WITH DIFFERENT AMOUNTS OF CALCIUM AND PHOSPHORUS IN SAME RATIO

No. Animals	Diet	Per Cent Ca	Per Cent P	Av. No. Carious Lesions	Av. Caries Score
15.....	1	0.25	0.25	24±2.4	47±15
15.....	2	0.50	0.50	21±1.4	33±11
15.....	3	1.00	1.00	18±3.5	21± 6
15.....	4	1.50	1.50	17±4.0	20± 4

STATISTICAL EVALUATION

Diet	Av. No. Carious Lesions	t	P*	Av. Caries Score	t	P*
1.....	24			47		
		4.0	<0.01		2.4	<0.02
2.....	21			33		
		3.2	<0.01		3.0	<0.01
3.....	18			21		
		0.1	>1.0		0.5	>0.6
4.....	17			20		

* Probability of a significant difference.

As the diet became less cariogenic by the addition of calcium and phosphorus while the Ca/P ratio remained constant, the results of these experiments confirm the conclusion deduced from indirect evidence in previous experiments, namely, that the cariogenicity of our high-sucrose diet is influenced by its calcium or phosphorus content and not by the Ca/P ratio. It is of interest to note that Harris and Nizel[5] also obtained high and low caries scores with the same Ca/P ratio in the diet.

In our earlier experiments it was found that when calcium was kept at a constant level, the addition of phosphorus, up to approximately 1 per cent concentration, rendered the diet less conducive to caries. Beyond this point, no additional effect was produced by further increment in the phosphorus content. Likewise, a concentration of aproximately 1 per cent in calcium appeared to be the critical level for maximum effectiveness in reducing the cariogenicity of the diet.

Upon comparison of the results of the present and our previous experiments, it was noted that when calcium and phosphorus were increased from 0.25 to 0.50 and 1.00

per cent, the caries scores were very close to those obtained when only the calcium or the phosphorus was increased from 0.25 to 0.50 and 1.00 per cent. The effects obtained by increasing either the dietary calcium or the phosphorus were therefore not additive when the concentration of both elements was increased simultaneously—for this we are unable at present to offer an explanation.

DISCUSSION

A review of the literature leads one to the general conclusion that the cariogenicity of experimental diets fed to rats or hamsters after tooth eruption may be influenced by the phosphorus or calcium content of the diet. There are, however, certain discrepancies that remain to be resolved.

Phosphorus.—It appears to be well established that Na_2HPO_4 is effective in rendering synthetic diets less conducive to dental caries. In the experiments of Constant, Sievert, Phillips, and Elvehjem,[6] the addition of 2 per cent Na_2HPO_4 to their cariogenic diet retarded the development of fissure caries in the cotton rat. Similar results were obtained in our laboratories with the albino rat. Increasing the phosphorus content of a high-sucrose diet without changing the calcium concentration led to a reduction in the incidence and extent of fissure caries. The extra phosphorus was provided by using a larger amount of a mixture of NaH_2PO_4 and Na_2HPO_4 in making up the salt.[1] The severity of smooth-surface caries also and the number of carious teeth were reduced by the addition of 1.5 or 2.0 per cent NaH_2PO_4 to the wheat-flour diet which produces this type of caries.[7–9]

However, when phosphorus is added to the diet in the form of insoluble salts, it is apparently not effective against cariogenesis. In McClure's experiments,[7] $CaHPO_4$ was not cariostatic. In keeping with these results are those of Buttner and Muhler,[10] who found that the insoluble calcium pyrophosphate ($Ca_2P_2O_7$) or the very slightly soluble dicalciumphosphate dihydrate ($CaHPO_4 2H_2O$) did not reduce the incidence of fissure caries when added to a coarse corn-particle diet. On the other hand, in unpublished data (cited by McClure[11]), Strålfors observed a reduction in the development of dental caries in Swedish school children when $CaHPO_4$ was incorporated in their noon lunch by way of phosphated bread flour and sugar. These results, however, as noted by McClure, were obtained on a rather limited caries examination and only a 9-month period of examination.

When NaCl was added to $CaHPO_4$ in McClure's experiments, a definite cariostatic effect was observed, although NaCl of itself proved to be ineffective in this respect. McClure believes that the inability of $CaHPO_4$ to produce cariostasis may be attributed to the insolubility of this salt and that its effectiveness when NaCl was added may have been due to a solubilizing action of the latter.

From the evidence available at the present time, one may conclude that soluble phosphates in the diet are cariostatic in rats and hamsters after the teeth have erupted and are fully calcified. This conclusion is further supported by the experiments of Mitchell, Helman, and Chernausek,[12] who found a reduction in hamster caries when either Na_2HPO_4 or $(NH_4)_2HPO_4$ was added to a caries-producing diet, and by those of Nizel, Keating, Sundstrom, and Harris,[13] in which the addition of metaphosphoric acid to the diet resulted in a marked reduction in the caries scores of hamsters.

Calcium.—In the experiments of Constant *et al.*,[6] previously referred to, a definite

decrease in fissure caries in the cotton rat resulted from the addition of 1 per cent $CaCO_3$ to the diet. This cariostatic effect of $CaCO_3$ became more pronounced when the concentration was increased to 2 and 3 per cent. Conversely, decreasing the calcium level from 0.56 to 0 per cent caused an increase in the incidence and extent of caries.[14] In experiments reported from our laboratory,[2] an increase in the calcium content of the diet from 0.29 to 0.57 and 1.01 per cent by the addition of $CaCO_3$, while the phosphorus content was kept at a constant level, led to a progressive decrease in the caries score. McClure,[7] however, found that $CaCO_3$ added to a wheat-flour diet was not cariostatic for smooth-surface caries. The discrepancy between McClure's results and those obtained in the Wisconsin and Emory laboratories may perhaps be accounted for by the fact that McClure was studying smooth-surface caries, whereas we, as likewise Constant and her associates, were concerned with fissure caries. Different mechanisms may possibly be involved in the production of these two types of caries and influenced differently by dietary factors. This supposition is supported by the fact that many diets which produces fissure caries do not produce smooth-surface caries.

There are, however, other discrepancies in the literature which cannot be explained in this way. Dalderup[15] found that the addition of $CaCO_3$ to a coarse corn ration or to a ricket-inducing ration was ineffective in reducing dental caries. In the experiments of Barnard and Johansen[16] the addition of 2 per cent $CaHPO_4$ to relatively low cariogenic diets effected a significant reduction in dental caries, whereas the same salt added to a severely cariogenic diet was ineffective in reducing the cariogenicity of the diet. In later experiments reported from Johansen's laboratory,[17] the addition of 2 per cent $CaCO_3$ to a cariogenic diet actually increased its cariogenicity, whereas the same amount of calcium acetate made the diet less conducive to caries. The discrepancies and apparent contradictions in the results obtained in different laboratories by adding calcium, particularly in the form of carbonate, to cariogenic diets pose a puzzling problem which can be solved only by further experimentation.

SUMMARY

The calcium and phosphorus concentration of our high-sucrose diet fed to albino rats was increased in equal amounts, thereby maintaining a constant Ca/P ratio.

With an increase in the calcium and phosphorus from 0.25 to 0.50 to 1.00 per cent, there was a progressive decrease in the number of carious lesions and the caries score.

The caries conduciveness of the diet was not affected by a further increase in the calcium and phosphorus concentration to 1.5 per cent.

These results confirm the conclusion deduced from previous observations, namely, that the cariogenicity of our high-sucrose diet is influenced by the actual amount of calcium and phosphorus in the diet and not by the Ca/P ratio.

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A Clinical and Experimental Study of Mucocele (Retention Cyst)

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Conflicting reports have appeared in the literature concerning the pathogenesis of mucocele or the so-called "retention cyst" of minor salivary glands. A commonly accepted hypothesis that mucoceles are due to accumulation of mucus in the salivary glands, the excretory duct of which has apparently been obstructed, has been challenged and replaced by a newer concept, first advocated in 1956 by Bhaskar, Bolden, and Weinmann.[1] This new concept is based on both clinical and experimental evidence. By definition, a cyst should be lined by epithelium, which Bhaskar *et al.* failed to see in any of the 19 surgically removed mucoceles. Similar findings were observed by Møller[2] and by Standish and Shafer[3]. Møller, in a report of 44 clinical mucoceles, found an epithelial lining in only 1 of 19 specimens which were examined histologically. Standish and Shafer noticed epithelial lining in only 4 of the 97 cases studied microscopically.

Bhaskar *et al.*[1] failed to produce mucocele by ligation of the excretory duct of the salivary glands, thus ruling out obstruction as a possible etiology of the experimentally produced lesions. This finding has been confirmed by Standish and Shafer. However, the former investigators claim to have successfully produced lesions similar to human mucoceles in rats and mice by cutting off the excretory duct of the salivary glands. No other study is available to substantiate this finding. The present investigation was undertaken to replicate their work.

MATERIALS AND METHODS

The present study was divided into two parts. The first part dealt with the gross, microscopic, and clinical observations on human mucoceles, and the second part was based on the experimental production of mucoceles in animals by various means.

Clinical study.—The material consisted of 66 surgically removed human mucoceles collected from the files of the School of Dentistry, University of Minnesota. The dental records of the patients with mucoceles were reviewed. The microscopic sections of the specimens were re-examined. When indicated, fresh sections were recut and stained with hematoxylin and eosin. Only in special instances were other stains employed.

Experimental study.—Forty-five male white rats, 4 months of age, were equally divided into three groups of 15 animals each. They were distributed in such a way

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that each group contained an equal number of animals of comparable weights. Each animal was anesthetized with 3.5 mg. of veterinary nembutal per 100 gm. body weight and was then maintained with ether. Through a ventral mid-line incision, the right submandibular gland was exposed and its ducts separated from the surrounding fascia. In the first group of animals the duct was ligated near its exit from the gland; in the second it was cut at the same point; and in the third group it was pinched with a hemostat. Five animals from Groups I and II were sacrificed at 3-, 7-, and 14-day intervals, respectively. In the third group, 5 animals were sacrificed at 1-, 3-, and 7-day intervals. The submandibular glands from these animals were removed and immediately immersed in 10 per cent formalin. They were processed for microscopic examination in a routine fashion and were stained with hematoxylin and eosin.

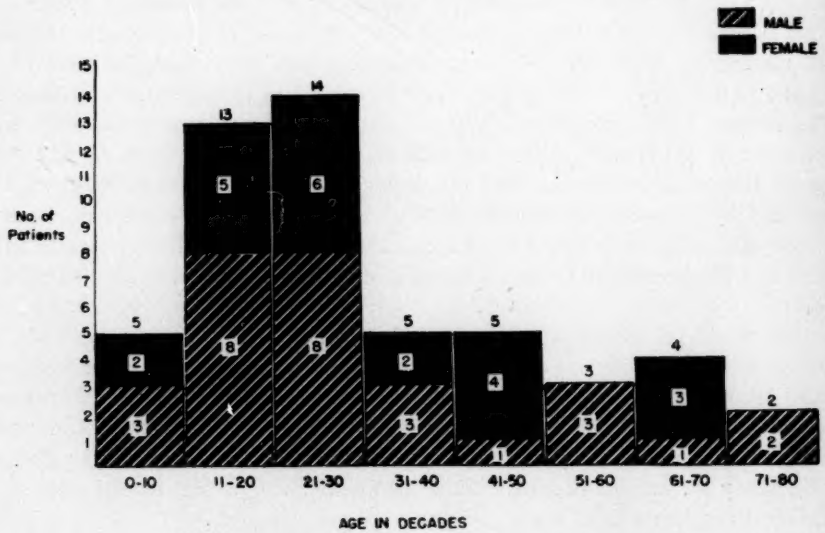

FIG. 1.—Age and sex distribution of 51 cases of mucoceles

RESULTS

Clinical observations.—The mucoceles were almost equally distributed between the sexes, 36 of them occurring in the males and 27 in the females, while in 3 cases information was not available. The age was known in 51 cases, of which 27 were in the second and third decades (Fig. 1). The youngest patient in the series was 5 years of age and the oldest 80, with an average age of 30.6 years. Of the 63 cases in which location was cited, 45 occurred in the lower lip, 7 in the buccal parieties, 3 in the alveolar ridge, 2 each in the palate, tonsillar region, and upper lip, and 1 each in the vestibule and floor of the mouth. The duration of the lesions was known in 20 patients and ranged from 1 week to 156 weeks, with a median of 10 weeks. The history was inadequate in most cases, so that no correlation could be established between trauma and appearance of the lesion. However, Standish and Shafer[8] have reported a history of obvious trauma in 11 per cent of their 97 patients.

Grossly, a typical lesion of mucocele on the lip was described as a round or oval,

smooth, fluctuant, translucent swelling with a bluish or greenish-blue tinge, especially when present immediately under the mucosa. When deep-seated, it was a firm, nodular, freely movable swelling which was difficult to differentiate from glandular hypertrophy. A history of spontaneous rupture, collapse, and refilling of the lesion was present in 6 patients. On cutting, soft and fluctuant lesions were usually unilocular and filled with thick gelatinous fluid which often escaped.

Microscopically, the mucoceles presented a wide variation in their structure. The most common variety of mucocele was characterized by the formation of a well-circumscribed, cystlike space surrounded by granulation tissue of varying degrees of maturity

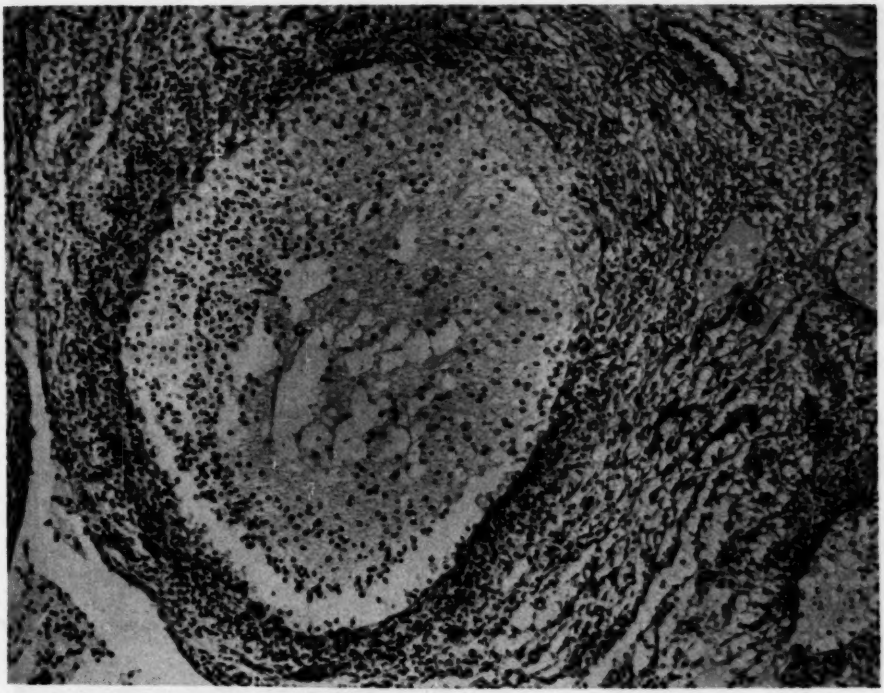

FIG. 2.—Photomicrograph of most common variety of mucocele, showing cystlike space surrounded by granulation tissue.

(Fig. 2). This space was usually filled with a homogeneous eosinophilic material in which were scattered inflammatory cells of different types, chiefly the large mononuclear cells. Occasionally, remnants of cords of epithelial cells were seen floating in the mucus pool (Fig. 3). When the mucus pool was localized superficially, it caused thinning of the overlying epithelium and loss of rete pegs (Fig. 4). In occasional cases, one could observe the relationship between the mucus pool, the feeding duct, and the attempt by the surrounding tissue to wall off the mucus pool by granulation tissue (Fig. 5). When the mucocele had ruptured spontaneously or had been of long duration, its walls were usually of thick granulation tissue with a small lumen with little or no visible mucin.

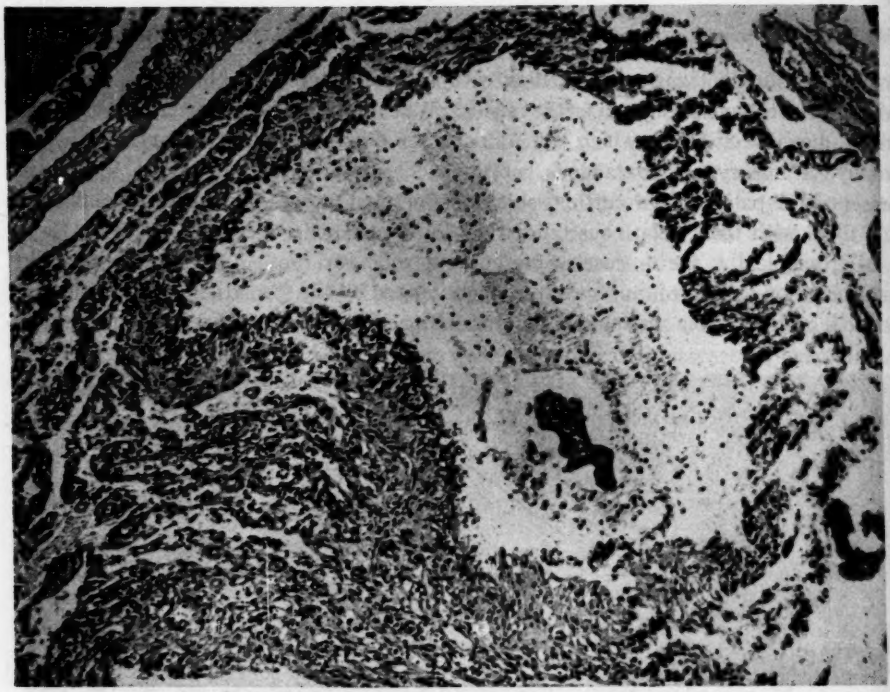

FIG. 3.—Photomicrograph of a mucocele. Note epithelial remnants floating in mucus pool

FIG. 4.—Photomicrograph demonstrating a superficially situated mucocele. Note loss of rete pegs

FIG. 5.—Photomicrograph, showing relationship between mucus pool, feeding duct, and granulation tissue.

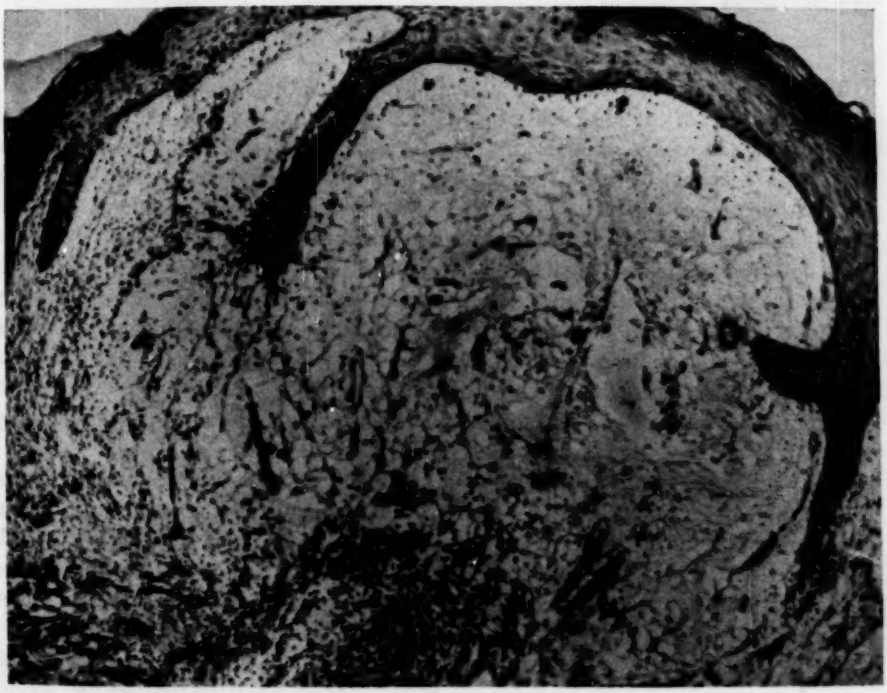

FIG. 6.—Photomicrograph, showing mucus interspersed throughout lamina propria and submucosa

Another variety was characterized by the presence of intercellular mucus throughout the lamina propria and submucosa (Fig. 6). In early lesions the mucus was intermixed with granulation tissue, whereas in long-standing cases fibroblastic activity was a predominant feature.

Ten cases in the present series were diagnosed clinically as mucoceles but were represented microscopically by a single large dilated duct with or without mucin in it. There was no evidence of spillage of mucin in the surrounding tissue, although inflammatory reaction and fibrosis around the dilated duct were present (Fig. 7).

Over 80 per cent of the specimens examined had minor salivary glands which were characterized by such secondary changes as atrophy of glandular parenchyma and its replacement by interstitial fibrosis and dilatation of the ducts (Fig. 8).

Experimental results.—In Group I animals, at 3- and 7-day intervals, the ligation of the excretory duct resulted in dilatation of the ductiles and their rupture, adenitis, necrosis, atrophy of the glandular parenchyma, and interstitial fibrosis. At 14 days, the glands were shriveled to a fibrous mass. In Group II animals, the changes at 3 and 7 days were quite dramatic. The glands were much larger than normal in size. They were soft, smooth, and shiny. When cut, they showed one or more areas of mucus accumulation (Fig. 9). Microscopically, they showed mucus intermixed with granulation tissue (Fig. 10) or mucus surrounded by granulation tissue not unlike human mucoceles (Fig. 11). However, no such lesions were demonstrable at 14 days, at which time the glands were mostly atrophic and replaced by fibrous connective tissue. In Group III animals, the changes at 1 and 3 days were comparable to those in the preceding group at 3 and 7 days. Nearly half the animals showed adequate evidence of mucocele-like lesions at 1 and 3 days but none at 7-day intervals.

DISCUSSION

The present clinical and experimental study confirms the findings of Bhaskar *et al.*,[1] as well as those of Standish and Shafer,[3] that a vast majority of mucoceles may be produced by the escape of mucus into the surrounding tissues from an excretory duct which has been mechanically traumatized. The mucus may be diffused within the surrounding tissues or form a discrete pool surrounded by a wall of connective tissue or granulation tissue with varying degrees of maturity. Subsequent changes in the glands, such as atrophy of the parenchyma, interstitial fibrosis, partial obstruction of the smaller ducts, and retention of mucus in them or their rupture from back pressure, are secondary to accumulation of mucus and inflammatory reaction. Complete obstruction does not seem to produce mucoceles, as is evident from experimental ligation of the excretory ducts of the submaxillary glands in rats. This is further supported by the fact that, of 3 cases of sialolithiasis of minor salivary glands seen in our laboratory, none had either gross or microscopic attributes of a mucocele.

The role of partial obstruction, however, has not yet been investigated. It appears that in some cases a partial obstruction of the excretory duct from a sialolith or from extra-luminal causes may produce distention of the duct or even its rupture and subsequent escape of mucus into the surrounding tissue, forming a mucocele. The authors have recently seen such a case of a sialolith of a minor salivary gland and a mucocele present in the same specimen (Fig. 12). Similarly, in 10 cases in our series, in which only a markedly dilated duct was seen with no evidence of spillage in the surrounding tissues,

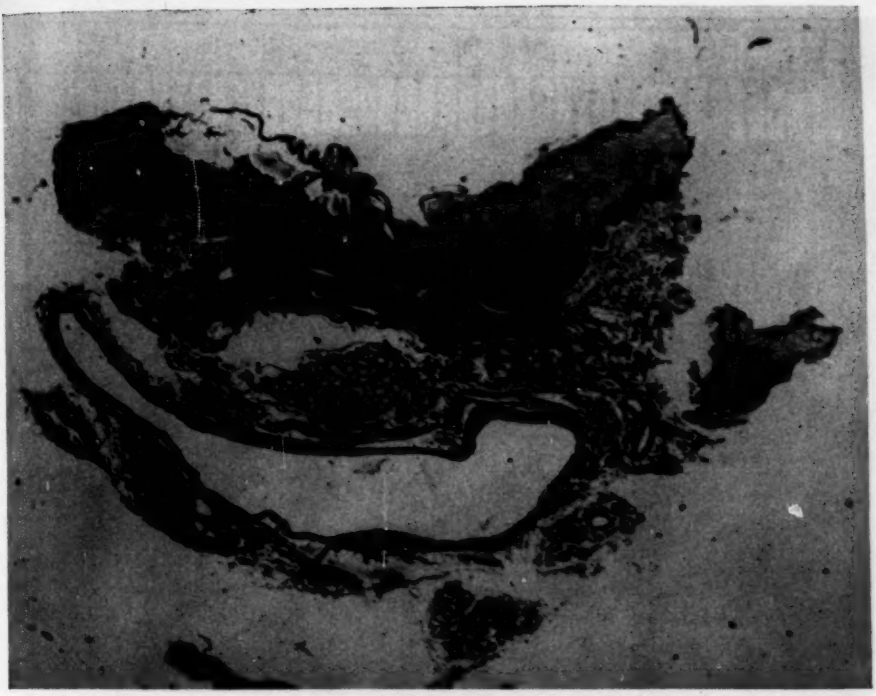

FIG. 7.—Photomicrograph, demonstrating another type of mucocele with no visible mucus spillage. Note dilated duct and inflammatory fibrosis surrounding it.

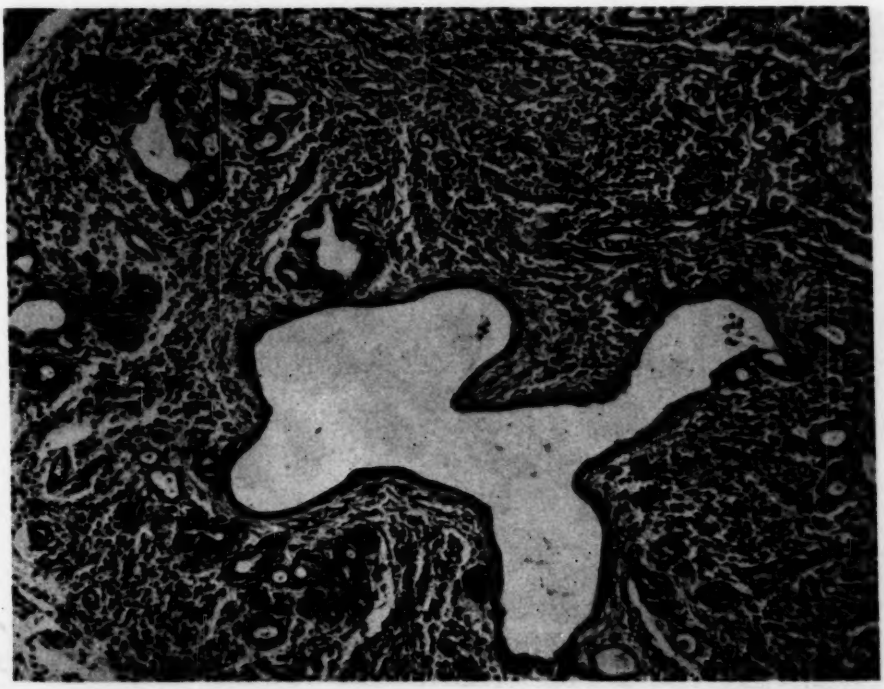

FIG. 8.—Photomicrograph, showing secondary changes in associated minor salivary gland. Note ductal dilatation, glandular atrophy, and interstitial fibrosis.

FIG. 9.—Gross appearance of an experimentally induced mucocele. Note the glistening areas of mucus accumulation.

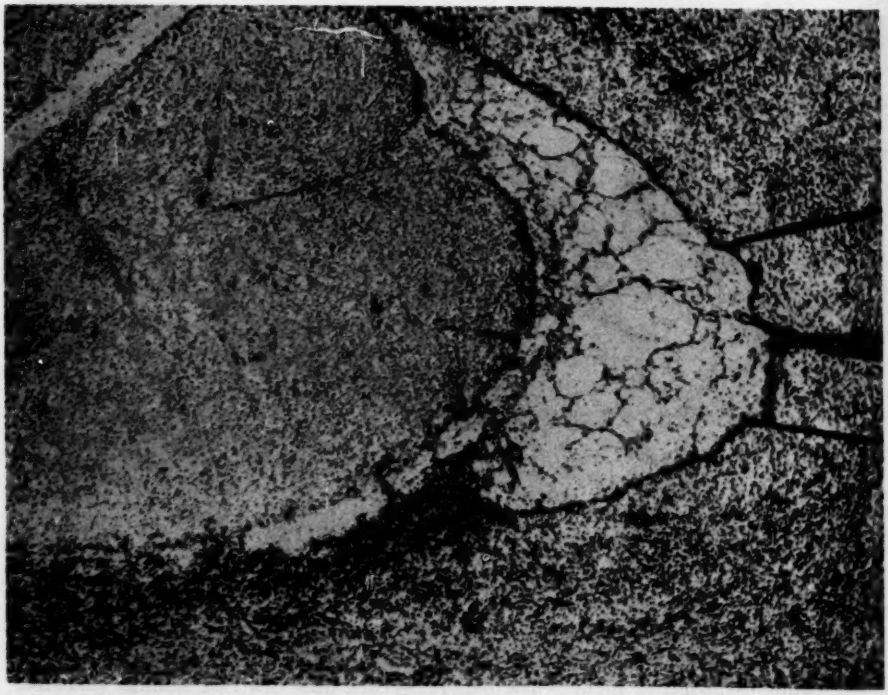

FIG. 10.—Photomicrograph of an experimentally induced mucocele. Note intermixing of mucus and granulation tissue.

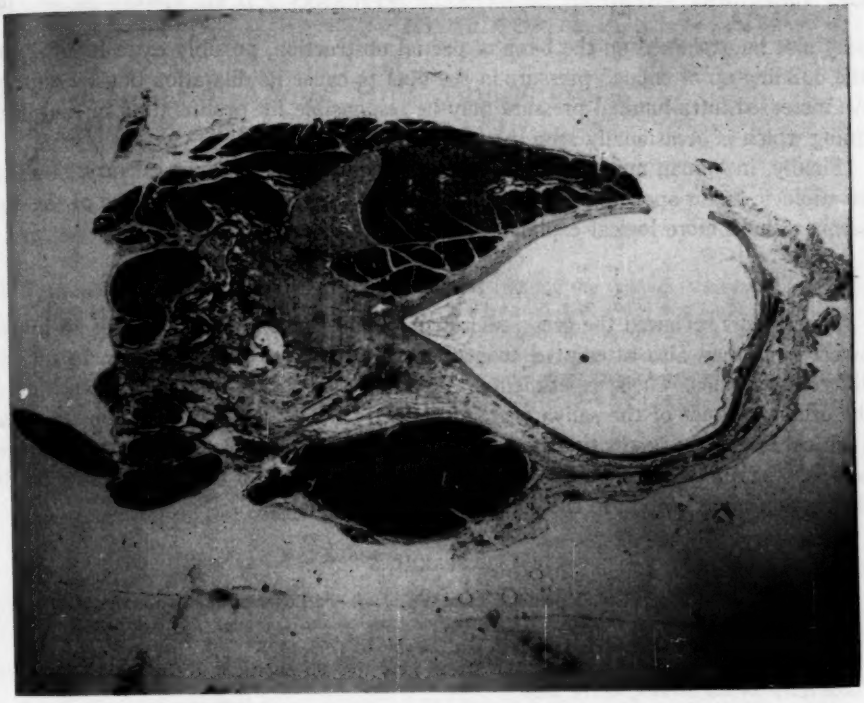

FIG. 11.—Photomicrograph of an experimentally induced mucocele in which mucus pool is surrounded by granulation tissue.

FIG. 12.—Photomicrograph, showing sialolithiasis and mucocele present within same specimen. The sialolith is shown in the duct on the right, the mucocele on the upper left of the illustration.

may also be explained on the basis of partial obstruction, possibly extra-luminal, and the building-up of enough pressure in the duct to cause its dilatation but not rupture. An increased intra-luminal pressure may be responsible for proliferation of the ductal lining which is occasionally seen in such cases.

Finally, in human mucoceles, a complete severance of the minor salivary glands as an etiologic factor appears a bit impracticable. On the other hand, pinching of the duct seems to be a more logical explanation for the formation of mucoceles.

SUMMARY

The authors reviewed the gross, microscopic, and the clinical features of 66 human mucoceles. They also attempted to produce such lesions experimentally in rats by different methods. Their experimental work indicates that severance or pinching of the excretory duct of the salivary glands rather than complete obstruction may play a critical role in the production of mucocele-like lesions. The role of partial obstruction, however, has not been investigated. It would appear that in occasional cases partial obstruction may also cause the formation of such lesions.

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Effect of Types of Alloys, Mold Temperature, and Casting Pressure on Casting Shrinkage

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Both direct and indirect methods have been used for determining the casting shrinkages of dental alloys. The direct methods used by R. L. Coleman (*D. Cosmos*, **68**:743, 1926) and G. M. Hollenback and E. W. Skinner (*J.A.D.A.*, **33**:1391, 1946) produced consistent and accurate data, but specimens of impractical sizes and forms were used. In indirect methods, casting shrinkages of inlays have been calculated from dimensional changes in castings and the investment expansions determined separately, but they generally lacked proper proof that investments expanded in casting rings in the same magnitude as those not in casting rings.

Fusayama has developed an indirect technique for determining with reliable accuracy casting shrinkage on specimens of sizes and forms representative of practical inlays or crowns. He found that casting shrinkage of regular inlay gold alloy (B and C types) was 2.1 per cent for simple internal inlays, 2.0 per cent for crowns, and 1.9 per cent for mesio-occluso-distal inlays (*J. Pros. Den.*, **9**:468, 486, 1959).

TABLE 1

CASTING SHRINKAGES AND LINEAR THERMAL EXPANSIONS OF VARIOUS ALLOYS

ALLOYS	A*	B†	C‡	D§	E‖	TYPE C GOLD ALLOY (PREVIOUS DATA BY FUSAYAMA)
Main constituents	Cu,Zn,In	Cu,Zn,Al	Ag,Pd,Cu,Au	Ag,Zn,Cd,Sn	Ag,Sn,Cd,Zn	
Melting range (° C.) liquidus	890	889	1,053	831	612	
Melting range (° C.) solidus	878	878	1,000	815	600	
Mold temperature (° C.)	700	700	700	700	500	700
Casting shrinkage (%) column	2.19(±0.03)	2.14(±0.05)	1.61(±0.04)	1.65(±0.09)	1.52(±0.05)	2.10
Casting shrinkage (%) crown	1.96(±0.06)	1.94(±0.08)	1.61(±0.01)	1.43(±0.04)	1.31(±0.04)	1.98
Casting shrinkage (%) M-O-D inlay	1.75(±0.02)	1.74(±0.05)	1.58(±0.05)	1.32(±0.02)	1.31(±0.02)	1.90
Linear therm. exp. (%)	2.24	2.14	1.76	1.64	1.38	.....
Softening point (° C.)	880	885	980	725	550	.....

* Progold, Hayashi Alloy Co.
† Orden, Ando Alloy Co.
‡ Arau-Cast, G. C. Chemical Mfg. Co.
§ Silvum, Shofu Dental Mfg. Co.
‖ G. C. Chemical Mfg. Co. (Japan).

In this study the same technique was used for determining the casting shrinkages of various inlay casting alloys other than gold and also for investigating the effect of mold temperature and casting pressure on casting shrinkage. Linear thermal expansions to the softening points of these alloys were also measured and compared with the casting shrinkages. Findings are summarized in the following.

As seen in Table 1, casting shrinkage was greatest in column, less in crowns, and smallest in M-O-D inlays, regardless of the alloy used. Casting shrinkage was less with alloys having a lower melting range and a lower thermal coefficient of expansion. Linear thermal expansion to the softening points was generally greater than the casting shrinkage of external inlays and slightly smaller or greater than that of internal inlays.

Lower mold temperature reduced the casting shrinkage only in column (simple internal) inlays but not in crowns (external inlays). Such a reduction is probably insignificant in clinical internal inlays, which are generally smaller or more complex than the column used in this test. Variation in casting pressure did not alter casting shrinkage in either columns or crowns (internal or external inlays).

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Presence of Se^{75} in Rat Saliva after Intracardiac Injection of Radioactive Sodium Selenite

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The present study was planned to determine whether or not the major salivary glands of the rat are permeable to selenium in the form of a buffered selenite administered directly into a heart chamber.

Nineteen male Sprague-Dawley albino rats, weighing approximately 250 gm., were ether-anesthetized and given intracardiac injections of 0.1 ml. of sodium selenite, phosphate-buffered to pH 6.8. Including the carrier selenium, each animal in a group received 37.5 mg of Se which contained 28 microcuries of radioactivity. At intervals of 2, 3, 5, 9, and 24 hours after isotope injection, each animal was anesthetized with nembutal (3 mg/100 gm) and then given pilocarpine nitrate, 5.0 mg/kg, subcutaneously. Following the injection of the sialagogue, saliva was collected from the anesthetized rats in test tubes, using a plastic animal holder. At the conclusion of a 10-minute collection period, each

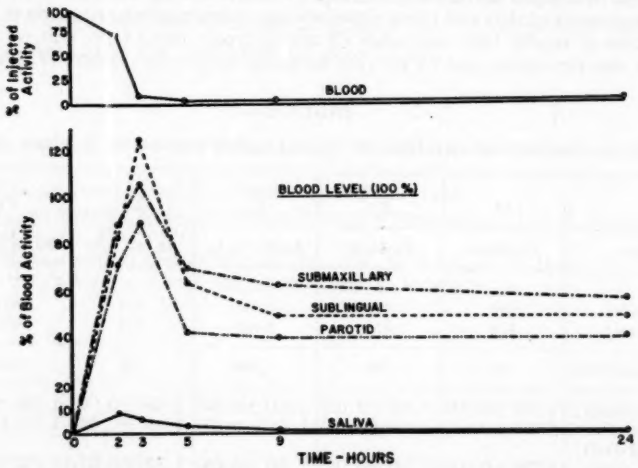

FIG. 1.—The relative amounts of Se^{75} present in blood, saliva, and salivary tissues after an intracardiac injection of $Na_2Se^{75}O_3$.

anesthetized animal was sacrified; a 2-ml. aliquot of blood was immediately aspirated from the heart and its radioactivity determined. During post mortem examination, the salivary glands were excised, blotte, and weighed prior to counting. In order to allow for any activity in these organs contributed by their blood content, individual glands from the first nine rats were homogenized and the hematocrit determined, This procedure demonstrated that the activity contributed by the blood retained in the tissues represented an insignificant fraction (less than 1 per cent) of the total activity and was omitted for the remainder of the experiment.

The upper curve in Figure 1 indicates the percentage of the injected dose of Se^{75} remaining per gram of blood, and the bottom curves in Figure 1 show the percentages of activity per gram of tissue or saliva when compared to the corresponding activity of the blood. Each point is an average value of all animals in that time period. Each group consisted of four rats, except that the 24-hour group contained only three. The maximum radioactivity of the mixed saliva occurred at 2 hours postinjection of the isotope. Our findings suggest that the salivary glands are permeable to selenium.

The question arises whether this element appears in the saliva as a transudate from blood plasma or whether it is resynthesized in the salivary glands into specific proteins, different from the plasma proteins which transport Se^{75} to the gland.

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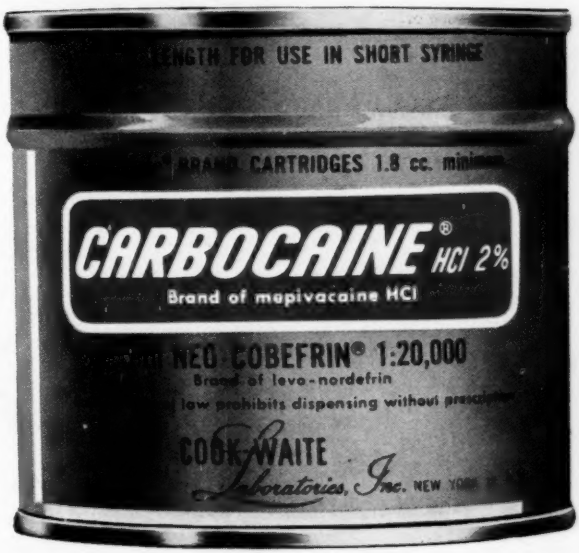

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